A CONTRIBUTION TO THE GENETICS OF THE MOSQUITO *AEDES AEGYPTI* (L.) WITH PARTICULAR REFERENCE TO FACTORS DETERMINING COLOUR

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George Anderson Hugh McClelland
B.A. (Hons.), Cambridge.

Department of Entomology
London School of Hygiene and Tropical Medicine

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ABSTRACT

A Contribution to the Genetics of the Mosquito *Aedes aegypti* (L.) with particular reference to factors determining colour.

by

G.A.H. McClelland.

The literature relevant to mosquito genetics is first reviewed.

In the present work, 51 different strains of *Aedes* aegypti have been examined for colour variation in particular. Methods of rearing, handling and routine are briefly described.

Colour of the dorsal abdomen was so variable, that a scheme was devised to classify it, according to paleness, in 37 grades and sub-grades. These could be assigned to 15 numerical colour values to enable the paleness of a population to be quantitatively defined. Photography of variants was adopted as a routine.

In Linkage Group I, two partially sex-linked factors controlling eye colour and one controlling abdominal colour were isolated, and 4 linkage distances determined. This is the first example of partial sex-linkage in *A. aegypti* and the first three-point linkage estimation in any mosquito. In Linkage Group II one new mutant controlling thoracic colour was isolated and its recombination with the previously

described *s* locus measured.

At least 4 mutant alleles are shown to occur at the *s* locus and one is identical with a gene previously ascribed to another locus. Two new mutants were isolated in Linkage Group III and the crossover distance of one measured from the previously described *blt* locus at which a second mutant allele was isolated. A further three potentially useful mutants were obtained and other variation mentioned.

The variation in abdominal colour in 39 strains is described and discussed. The frequency of genotypes in populations polymorphic for an *s* allele suggests, though not significantly, some degree of heterosis.

Hybrids were successfully obtained between three pairs of *Stegomyia* species. The relationship between *A. aegypti* and *A. mascarensis*, one of the two crosses giving fertile hybrids, is discussed more fully.

A number of gynandromorphs and intersexes is also recorded.

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INTRODUCTION

INTRODUCTION

The application of genetics to the study of mosquitos has been surprisingly limited, considering the wealth of other information on the group and their comparative affinity to Drosophila. Perhaps this is because the study of disease vectors has been largely the preserve of medicine where genetics has, until recently, received scant attention. Mosquito genetics has nevertheless become, during the last decade, one of the more active growing points in medical entomology. A great impetus derived from the development, in many species, of resistance to insecticides and consequently the harsh realization of the genetically dynamic potential of insect populations.

To lend perspective to the present study, it is useful to review the gradual development of mosquito genetics from its origins to the present. The first phase probably ended with the comprehensive review by KITZMILLER (1953) who drew attention to the existence of a genetical aspect in mosquito studies and the urgent need for its further emphasis. Subsequent advances have been well summarized by ROZEBOOM and KITZMILLER (1958) and DAVIDSON and MASON (in press), so that anything beyond an outline treatment would here be superfluous.

THE GENETICAL ASPECT IN MOSQUITO STUDIES

CYTOLOGY

The earliest work on mosquito chromosomes stemmed from the great surge of interest in comparative cytogenetics following the rediscovery of Mendel's work, rather than from any special curiosity for mosquitos in particular. Over fifty years ago STEVENS (1910, 1911) examining gonial material, gave six as the diploid number of chromosomes in Culex, Culiseta and Anopheles. Stevens' figures of metaphase chromosomes could be little bettered today, but she was certainly looking at Anopheles when she described heterosomes formed by fusion of large equal and small unequal chromosomes (STEVENS, 1910). Her later comment on the apparent absence of heterosomes in Culex and Culiseta (STEVENS, 1911) was to receive genetic confirmation thirty-five years later (GILCHRIST and HALDANE, 1946).

The lower estimates of chromosome number by LOMEN (1914) and M. TAYLOR (1914) in Culex pipiens were attributed by METZ (1916) and WHITING (1917) to poor fixation and misinterpretation of the close chromosomal pairing characteristic of the Diptera. The same is probably true of CARTER's (1918) preparations of Aedes aegypti. All more recent studies have confirmed a diploid number of six. This is the lowest chromosome number in any group of the cytologically-known Metazoa given by MAKINO (1951) with the

exception of some Platyhelminthes, Nematoda, Nematomorpha, Polychaeta, a few lower Arthropoda and some anomalous Hemiptera.

In the Culex pipiens group, which has been most extensively worked, it is generally agreed that there are two equal pairs and one smaller pair of metacentric chromosomes (HANCE, 1917; MOFFETT, 1936; SUZUKI, 1939; CALLAN and MONTALENTI, 1947; KITZMILLER and FRIZZI, 1954; BRELAND, 1961; RAI and CRAIG, 1961). At prophase the centromeres appear as "flared" unstained regions (GRELL, 1946b; BRELAND, 1961). Meiotic and mitotic preparations are usually obtained from gonial and other tissues of 4th stage larvae or pupae, but LONG (1961) claims confirmatory mitotic figures from 1st stage larvae.

One of the large pairs of chromosomes in A. aegypti is said by RAI and CRAIG (1961) to be submetacentric with an arm length ratio of 4 : 3. The same authors report that the chromosomes of Aedes vexans, Aedes atropalpus, Aedes stimulans and Aedes albopictus resemble those of the C. pipiens group. SUZUKI (1939) however observed three pairs of different length in A. albopictus, as did KITZMILLER and FRIZZI (1954) in Aedes geniculatus. BRELAND (1961) in the most extensive survey, of 24 species in 9 genera, considers that there is little likelihood of much metaphase variation between species of mosquito outside the anophelines. In this tribe, although the two larger pairs are typically V-shaped metacentrics, the

distinct heterosomes vary from punctiform in Anopheles claviger (FRIZZI, 1950b) to subtelocentric and about half the length of the autosomes in Anopheles maculipennis (FRIZZI, 1950 a,b). The difference between the X and Y chromosomes in the male is most marked in Anopheles stephensi (RISHIKESH, 1959).

HOLT (1917) discovered the developmental polyploidy in the pupal gut of C. pipiens, but it was left to BERGER (1937, 1938) to show that this was followed, at metamorphosis, not by cytolysis but by the unusual process of somatic reduction division. The few large cells of the larval gut with 48, 54 or more, chromosomes thereby divide into the 8 or more times as many small diploid cells that form the adult gut. GRELL (1946a) confirmed Berger's work with detailed descriptions of the processes of both duplication and reduction. SCHUH (1951) showed that, by preventing the reduction division, colchicine arrested metamorphosis. RISLER (1959, 1960) has recently described somatic reduction division in A. aegypti with few points of difference.

Studying meiotic figures in C. pipiens, MOFFETT (1936) noted wide variation in chiasma frequency between individual mosquitos. The bivalents of the short chromosome rarely had more than one chiasma. Where two chiasmata occurred in a bivalent, these were most often both terminal. This was interpreted by PATAU (1941) as evidence of chiasma interference across the centromere, a conclusion fully

confirmed by CALLAN and MONTALENTI (1947). Chiasma frequency in the small chromosome bivalent was 1.95, in the larger two bivalents 2.91. The chance of a proximal chiasma in one arm increased the more distal was a chiasma in the other arm. In contrast the same authors found no such interference in Culiseta longiareolata.

The existence of polytene chromosomes in mosquitos was first reported by BOGOJAWLENSKY (1934) in A. maculipennis and BERGER (1936, 1937, 1938) in C. pipiens, but SUTTON (1942) was the first to describe characteristic banding patterns similar to those of the familiar salivary chromosomes of Drosophila and some other Diptera. Working with C. pipiens and A. aegypti, Sutton's best preparations were from the malpighian tubes of 4th stage larvae, pupae or adults, but she was also able to use nuclei of the salivary glands, gastric caeca and mid-gut of the prepupal stage. To these possibilities, GILLHAM (1957) added cells of the rectum and anal papillae. Neither he nor STALKER (1954) were able to observe banded polytene chromosomes in the ovarian nurse cells or other adult genital tissue.

The construction of salivary chromosome maps in mosquitos was pioneered by FRIZZI (1947, et seq.) in his studies of species in the A. maculipennis group. FRIZZI and RICCIARDI (1955) made maps for the neotropical Anopheles albimanus and Anopheles aquasalis. Together with Holstein, Frizzi mapped the important African malaria vector Anopheles gambiae observing great inversion variability (FRIZZI and HOLSTEIN, 1956 and, with Kitzmiller, the chromosomes of the N.American

Anopheles punctipennis (FRIZZI and KITZMILLER, 1959). KITZMILLER and FRENCH (1961) have preliminarily reported a study on Anopheles quadrimaculatus and HOBBS (1962) has mapped the salivary chromosomes of several strains of A. albimanus for comparison. Other cytological studies of Anopheles will be mentioned later in connection with insecticide resistance.

Mapping of salivary-type chromosomes in Culicine species has lagged behind that in Anophelines, but a beginning was reported by KITZMILLER and CLARK (1952) in C. pipiens pipiens and C. pipiens fatigans. KITZMILLER (1954) later observed that good spreads were more difficult to obtain in C. pipiens molestus than in the other two species of the group, although there were no differences in banding pattern apparent between the three species. The chromosomes are not united in a chromocentre as in Anopheles but spread separately or even unite terminally to form rings. If bulbous, weakly staining regions are homologous with the centromeres, two of the chromosomes are obviously submetacentric. There are no differences that can be correlated with sex. The salivary chromosomes of A. aegypti have been described as very long and fragile by ALDIGHIERI (1961), who gives a photograph showing clear banding of chromosome fragments but no map. A preliminary map is said, however, to have been prepared by MESCHER (1960). Of all mosquitos so far studied, A. aegypti would seem to have the least amenable salivary chromosomes.

SPECIATION AND HYBRIDIZATION

The ultimate definition of a species is at the genetic level (MAYR, 1948) and it was largely as a taxonomic tool that genetics first proved its usefulness to the mosquito worker.

Anopheles

The phenomenon of "Anophelism *sine* malaria", the absence or decline of malaria in areas where the principal vector *A. maculipennis* was abundant, led ROUBAUD (1920), WESENBERG-LUND (1921) and GRASSI (1921) independently to suggest that the habits of this mosquito might vary in different places. This they linked with agricultural, social and economic changes that had resulted in a rising cattle population which might have diverted the mosquitos from man. An alternative suggestion was of the prior existence or induction of a race of *A. maculipennis* preferring cattle. In Holland VAN THIEL (1926) discovered statistical differences in the morphology of adult *A. maculipennis* reared from brackish sites compared with those from fresh water. He later (VAN THIEL, 1927) concluded that the differences were not the result of salinity and named the brackish-water form, which were short-winged, *atroparvus*, and the fresh-water form, which were longer-winged, *messeae*. DE BUCK, SCHOUTE and SWELLENGREBEL (1927, 1930) correlated the presence of the short-wing form with the malarious zones of Holland. Both forms were equally susceptible to the parasite, but the long-wing form hibernated and so did not

transmit malaria during the winter. The true-breeding of the short-wing form even after several generations in fresh water confirmed that the differences were genetic. The joint work of Hackett, Missiroli and Martini (MARTINI et al., 1931; HACKETT et al., 1932; MISSIROLI et al., 1933) showed that differences in the exochorion of eggs, described by FALLERONI (1926) were more reliable means of separating several forms of A. maculipennis than van Thiel's statistical characters. There was again a clear correlation between the presence of some of these forms, salinity of breeding place, degree of man-biting and incidence of malaria. ROUBAUD et al. (1933) showed that the ability to mate in a confined space, stenogamy, characteristic of atroparvus (ROUBAUD, 1932), behaved as a Mendelian dominant in crosses with the eurygamic messeae. The Dutchmen, continuing their careful work, demonstrated partial or complete sterility in crosses between atroparvus, messeae and some other forms (DE BUCK et al., 1934; DE BUCK and SWELLENGREBEL, 1935, 1937). The claim by ROUBAUD et al. (1937), on the basis of egg characters, that natural hybrids occurred in parts of France between atroparvus- and messeae-like forms has never been substantiated. It is probable that their strains were impure, and it must be emphasized that the exochorionic structure of the egg is determined maternally, independantly of the zygotic genotype.

CORRADETTI (1934 a,b, 1937 a,b) in Italy and BATES (1939) in Albania extended the work to the A. maculipennis of

southern Europe, clearly indicating the specific status seekers of the different forms. BATES and HACKETT (1939) and BATES (1940) present a complete picture. Crosses of males of A. atroparvus, the only stenogamic species, with females of six of the other species revealed varying degrees of infertility. A. messeae gave no viable F_1, A. sacharovi gave only sterile males, A. maculipennis (= typicus) gave an F_1 of both sexes, all sterile. The F_1 progeny of the other three species, A. subalpinus, A. melanoon and A. labranchiae, however, though mainly sterile, included a few fertile females which on repeated backcrossing to A. atroparvus yielded fertile males by about the third generation.

FRIZZI (1950 c) has recently succeeded in crossing female A. atroparvus with A. maculipennis using an entire room to overcome the barrier of stenogamy; the F_1 were all sterile females. He also observed inversions and asynapsis in spermatogenesis in the sterile males of the reciprocal cross. Later FRIZZI (1958) was able to cross A. messeae with A. maculipennis using a force-mating technique of McDANIEL and HORSFALL (1957). It is to be hoped that this technique, perhaps with the modifications that BAKER et al. (1962) suggest, may permit other possible crosses, as they have done with American species.

BURGESS (1948), ROZEBOOM (1952) and BARR (1954) have shown different extents of intersterility between members of the North American "maculipennis" group. In only one out of

eight crosses were no viable eggs produced. BURGESS (1955) succeeded in crossing A. freeborni with the less closely related species, A. punctipennis. About 10 percent of the eggs laid in each reciprocal cross hatched, but many of the larvae were abnormal and only one adult was obtained from 250 hatching eggs. Males of A. quadrimaculatus, A. freeborni or A. aztecus showed incomplete, though significant, discrimination for females of their own species when given a choice of another, except male freeborni which were unable to discriminate between their own females and those of aztecus (ROZEBOOM, 1952, 1953). The fact that sterility is neither complete nor as constant as in the case of the palearctic species is probably a reflection of the greater degree of geographic isolation between the American species.

Crosses between A. atroparvus of Europe and the American A. quadrimaculatus have produced some sterile females (MARYON et al. 1951). This was confirmed by FRIZZI (1954b), who was also able to raise hybrid larvae from reciprocal crosses of A. atroparvus with the American A. freeborni, and of male A. freeborni with the European A. maculipennis and A. subalpinus. Cytologically there was a noticeable lack of proper synapsis in these hybrids.

The example of A. maculipennis stimulated analysis of other species complexes in mosquitos. SWEET and RAO (1938) demonstrated partial sterility between two races of A. stephensi in India. ROZEBOOM and KNIGHT (1946) drew

similar conclusions from preliminary observations on the Anopheles punctulatus group of the western Pacific. REID (1962) emphasizes the need of simple mating tests to establish relationships within the Anopheles barbirostris complex of S.E.Asia.

The variety melas of A. gambiae was considered a separate species by RIBBANDS (1944 a,b) and MUIRHEAD-THOMSON (1945) in West Africa. A. melas bred in salt water and was a less potent malaria vector than the fresh-water A. gambiae (the converse of A. atroparvus and A. messeae). Crosses between the two forms yielded an F_1 of sterile males, with or without a proportion of normal females (MUIRHEAD-THOMSON, 1948; BURGESS, 1961). On the other hand HOLSTEIN (1960), in a preliminary study, found no differences in the banding pattern between A. gambiae and A. melas and concluded that the latter is merely a variety. MUIRHEAD-THOMSON (1951) later described a salt-water race of A. gambiae in East Africa, which may or may not be a homologue of A. melas. The whole problem in A. gambiae is receiving much attention at present. From the little published it would seem that all populations studied fall into one or more mating types between which crosses give normal females and sterile males (DAVIDSON, 1958a; DAVIDSON and JACKSON, 1962). The sterility is reciprocal, but backcrossing females to either parental strain males gives 50% sterile male offspring suggesting that the mechanism involved is genic rather than chromosomal. Attempts to separate

populations of A. gambiae by a statistical measure of maxillary index (HOLSTEIN, 1954) have not proved very useful (GILLIES and SHUTE, 1954; GOMA, 1961).

Culex

Parallel to the early work on A. maculipennis was the recognition of a species complex in the common house mosquito C. pipiens. Lacking the incentive that malaria gave to the study of Anopheles, that of Culex has developed slowly, but priorities are now changing with the growing importance of filariasis control.

FREEBORN (1926) drew attention to intergrades between C. pipiens and the tropicopolitan C. p. fatigans in that part of California where these supposedly distinct species overlapped. While searching for diets other than blood on which C. pipiens would mature eggs, HUFF (1929b) had accidentally discovered that some females showed autogeny in that they laid an egg raft without taking any food. A year later ROUBAUD (1930) suggested the existence of a distinct race of C. pipiens in Europe differing in showing autogeny, in its ability to mate in a small space (stenogamy), breeding through the winter without diapause (homodynamy) and preference for human rather than avian blood.

ROUBAUD (1929) had earlier cited an example of an ornithophilic autogenous strain, LAVEN (1951a) has pointed out that there is no real evidence that the biological characters

are linked; certainly CALLOT and DAO VAN TY (1943) record a stenogamic anautogenous strain, KITZMILLER (1952) another such strain that is also homodynamic and SPIELMAN (1957) an autogenous heterodynamic strain. Nevertheless, as MATTINGLY (1951) points out and SHUTE (1951) clearly demonstrates, Roubaud's four characters together form an integrated adaptation to the urban habitat.

MARSHALL and STALEY (1937) attempted to define the morphological differences between C. p. pipiens and the autogenous form which they named molestus; the usefulness of their distinctions has not been borne out by CALLOT (1947, 1954, 1955) in analysis of the two forms and their hybrids. In contrast the genetic basis of the biological characters seems to be clearer. It is most generally agree that autogeny is recessive (ROUBAUD, 1930; TATE and VINCENT, 1936; CALLOT, 1947, 1955, LAVEN, 1951 b). The indication in DE BUCK's (1935) work of partial dominance probably implies heterozygosity in his original material. This cannot explain the clear dominance found by KRISHNAMUETHY and LAVEN (1961) in crosses of C. p. fatigans, in which autogeny is unknown, to autogenous C. p. molestus. In the reciprocal cross WEYER (1936) had claimed maternal inheritance for autogeny. SPIELMAN (1957) considered the control of autogeny to be bifactorial and partially sex-linked. Absence of autogeny in the F_2 from an anautogenous x autogenous cross (VINCENT, 1933) probably reflects inadequate larval nutrition

or other extrinsic factor. Partial penetrance of a single gene for autogeny is the plausible explanation of some of these anomalies offered by KITZMILLER (1953). Stenogamy has always behaved as a dominant character on crossing with eurygamic forms (VINCENT, 1933; DE BUCK, 1935; WEYER, 1935; TATE and VINCENT, 1936; CALLOT, 1947).

Probably the clearest distinction between C. p. fatigans and C. p. pipiens is the ratio of the distance between the dorsal and ventral arms of the phallosome (DV) to the spread of the dorsal arms (D). This ratio was used initially by SUNDARARAMAN (1949) to demonstrate possible natural hybridization in the U.S.A. The DV/D ratios of northern pipiens were all much lower than those of southern fatigans, but the intermediate ratios in the region of overlap were identical to those of laboratory hybrids. Fully fertile hybridization between fatigans and pipiens or molestus from different areas has also been claimed by WEYER (1936), FARID (1949), KITZMILLER (1950), BARR and KARTMAN (1951), KNIGHT (1953) and ROZEBOOM (1958). It seems from these reports that the DV/D ratio is polygenically controlled, since reversion to parental-type ratio occurs with repeated backcrossing. In other cases, partial or non-reciprocal sterility is reported (ROUBAUD, 1941; LAVEN and KITZMILLER, 1954; DOBROTWORSKY, 1955; CALLOT, 1955; PAL and KRISHNAMURTHY, 1958; KRISHNAMURTHY and LAVEN, 1961). Differential mating activity in the three forms seems also to lead to sexual isolation

(ROZEBOOM and GILFORD, 1954b).

Although the crosses of pipiens x molestus mentioned earlier in connection with autogeny were all fertile, a later cross made by ROUBAUD (1933) was only fertile using molestus females. LAVEN and KITZMILLER (1954) found an American pipiens completely intersterile with two German strains of molestus yet fertile with a third German strain. SIMONETTI (1952) in Italy failed to cross female molestus from Rome with pipiens from Tuscany, while succeeding in the reciprocal.

Sterility is not confined to crosses between the three forms. Within fatigans, complete fertility between strains from Lagos (West Africa) and South America (SERVICE, 1956), and between Brazzaville (West Africa) and a South Pacific island (ROUBAUD, 1956), contrasts with the non-reciprocal sterility of the latter two strains when crossed with a third African strain from Dakar (ROUBAUD, 1956).

It is, however, the occurrence of non-reciprocal sterility between strains of molestus that has attracted most attention. MARSHALL (1938) made all possible matings between three English strains - from Hull, Hayling Island and Westminster - and one from Paris. The Hull and Paris strains were fully interfertile and were fertilized by Hayling or Westminster males. Hayling and Westminster females were not fertilized by Hull or Paris males and the cross between these former strains only succeeded using Westminster females. ROUBAUD (1941) observed some infertility between strains from

Paris and Tunis, non-reciprocal sterility between two French strains (ROUBAUD, 1945) and, later, complete fertility between other French strains and one from Corsica (ROUBAUD and GHELELOVITCH, 1950). GHELELOVITCH (1952) attempted all crosses between strains from Hamburg, Paris and Tunis. Sterility was complete between those from Hamburg and Paris, non-reciprocal between those from Paris and Tunis and absent between those from Hamburg and Tunis. Differential sterility in reciprocal crosses between two Italian strains is reported by D'ANCONA (1962 a,b). Cytoplasmic inheritance was first suggested as a possible explanation of these sterility phenomena by GHELELOVITCH (1952) but the credit for a deeper understanding of the problem goes to LAVEN (1951, 1953 et seq.). Repeating some of the earlier crosses he tested a total of 17 strains, including a strain each of pipiens and fatigans from America. These fell into 9 distinct mating types, between which were made all possible intercrosses (LAVEN 1957b), showing complex sterility relationships.

Laven has concentrated his final analysis on the crosses between the strains from Hamburg (Ha) and Oggleshausen (Og) in north and south Germany respectively. Ha females are fully fertilized by Og males, but the reciprocal cross is completely sterile except for very few eggs which give rise only to females. LAVEN (1956a, 1957b) has demonstrated, with the aid of dominant markers, that these are cases of induced parthenogenesis. Male hybrids from the Ha x Og cross are,

like Ha males, incapable of fertilizing Og females. Throughout 52 repeated backcrosses of hybrid females to Og males, the hybrid males retained the Ha mating type. The hybrid females similarly retained full fertility on crossing with Ha males. SMITH-WHITE (1950) had pointed out, in connection with Aedes scutellaris, vide infra, that repeated backcrossing of this sort was necessary to distinguish cases of genome-cytoplasm incompatibility from true cytoplasmic inheritance. In the former case the gradual substitution of Og for Ha chromosomes would have led to a gradual lowering of sterility between the hybrid male and Og female. Laven used a dominant marker gene to confirm that Og genes were in fact transferred to the hybrids.

The essence of Laven's argument (LAVEN, 1957b) is that a plasmagene, carried in the middle piece of the Ha spermatozoon, is incompatible with Og cytoplasm, but that the Ha egg cytoplasm containing the plasmagene is unaffected by the entry of the middle piece of the Og spermatozoon. This small amount of Og cytoplasm is presumably destroyed and not incorporated in the Ha cytoplasm, otherwise 52 repeated doses might be expected to have modified the Ha cytoplasm. Although the possibility that a virus-like agent is involved is not disproved, Laven considers it most improbable. Purely maternal inheritance, of which this is a beautifully demonstrated example, is a rare phenomenon (CASPARI, 1948).

LAVEN's (1957b) suggestion that this type of non-

reciprocal, cytoplasmic inherited, sterility may lead to partial isolation and genetic differentiation of local populations, has been criticized by CASPARI and WATSON (1959) who consider that the cytoplasm of one of the two strains is certain to be eventually eliminated if the two meet and hybridized in nature. Where the selective values of the two cytoplasms are equal, the cytoplasm of the strain with the incompatible females (i.e. Og) will be eliminated. If this cytoplasm had a selective advantage over the cytoplasm of the other strain (Ha), elimination of either cytoplasmic type is possible depending on the initial frequencies of the two strains. The condition of equilibrium is metastable. The relative gene frequency of the final population will however be the same as that of the initial populations combined. According to Löven (personal communication), this is actually happening in Germany and the Ha type is gaining ground from the Og type.

It is interesting that in south-east Australia, where C. p. pipiens is replaced by C. p. australicus, similar patterns of non-reciprocal sterility occur between it and Australian strains of fatigans and molestus (DOBROTWORSKY and DRUMMOND, 1953) and within 5 strains of molestus (DOBROTWORSKY, 1955). More remarkable are the laboratory hybrids of either fatigans or molestus with Culex globocoxitus which has grossly distinct male genitalia (figured by MATTINGLY 1956). The intermediate genitalia of the latter hybrid were

indistinguishable from those of a wild-caught male which strongly suggests natural hybridization (DOBROTWORSKY, 1952).

It is evident that, within the C. pipiens complex, cytoplasmic sterility factors and the several genetic factors controlling morphological and biological characters are all independent of one another. Any attempt to define species based on these criteria is thus bound to break down beyond narrow geographical limits. This is in striking contrast to the anopheline examples discussed above. The C. pipiens complex, still a genetically open system, is perhaps at an earlier evolutionary stage.

Aedes

The subgenus Stegomyia of Aedes is one of the most distinctively ornamented groups of mosquitos and instances of taxonomic confusion are few. Thus in contrast to Anopheles and Culex, the geneticist's task is to confirm, rather than add to, the existing divisions.

The Aedes scutellaris group, scattered among many Pacific Islands, is one in which much speciation, born of geographical isolation, can be expected. WOODHILL (1949, 1950) justified his creation of a new subspecies A. scutellaris katherinensis by demonstrating its non-reciprocal sterility in crosses to the type species. Male A. s. katherinensis can be crossed with A. s. scutellaris to give fertile offspring. The F_1 retains the maternal mating type so that F_1 males are only

fertile to scutellaris females, although the F_1 females can be fertilised by both parental males. SMITH-WHITE (1950) explains this by postulating that, whereas scutellaris genome is inviable in katherinensis cytoplasm, katherinensis genome is compatible with scutellaris cytoplasm. He suggests that repeated backcrossing of the hybrid female to katherinensis males will lead to gradual substitution of katherinensis genes for those of scutellaris, so that subsequent male hybrids will be increasingly compatible with katherinensis females. SMITH-WHITE and WOODHILL (1954) set out to test the hypothesis and found no such reduction in sterility. The mechanism involved resembles that in C. p. molestus rather than Anopheles.

A. s. scutellaris also showed non-reciprocal sterility when crossed by PERRY (1950) to a sympatric population of Aedes pernotatus, the cross only succeeded using scutellaris females to give an entirely female F_1 resembling the mothers. These gave, on backcrossing to pernotatus males, a female and 4 males with pernotatus-type genitalia.

WOODHILL (1950) also showed that, while the Fijian A. pseudoscutellaris is completely sterile with both A. s. katherinensis and A. s. scutellaris, it is reciprocally fertile with A. polynesiensis from Tahiti (WOODHILL, 1954), but both he and ROZEBOOM and GILFORD (1954a) who also made the latter cross using Samoan polynesiensis found some lowering of fertility, especially with polynesiensis males.

Both workers crossed allopatric populations, the two subspecies occur together on Fiji and might be expected to show some sterility or other reproductive isolation.

Among African Stegomyia there may well be biological validity for separating as full species the closely similar Aedes africanus, Aedes luteocephalus and perhaps Aedes pseudoafricanus, although MATTINGLY and BRUCE-CHWATT (1954) were unable to test cross-mating between the latter two. The recent description of a fourth species, only minutely different from the others and of apparently similar habits (CORBET and VAN SOMEREN, 1962), serves to emphasize the need for genetic criteria.

Early attempts to characterize geographical races of A. aegypti failed to reveal any clear-cut differences (HOFFMANN, 1928; BRUG, 1928; MATHIS, 1934), but MATTINGLY (1957) has put a more convincing case for distinguishing a dark feral subspecies formosus in Ethiopian Africa from paler forms found there and elsewhere (vide infra). ALDIGHIERI et al. (1961a, 1961b) have used the confidence limits for three parameters, calculated from less than 50 specimens as co-ordinates for a three dimensional representation of several strains. Their results, to which they impute great significance, probably reflect no more than random variation of polygene frequencies between isolated populations. This is an example of a misleading answer given by the inappropriate use of multivariate statistical analysis in biological

research (HATHEWAY, 1962). Such an approach may be contrasted with the analogous but more elegant concept of taxonomic distance (SOKAL, 1961). The methods of numerical taxonomy (SNEATH and SOKAL, 1962) seem ideally suited to analysis within a group like Stegomyia.

CRAIG et al. (1961) have emphasized that the morphological diversity of A. aegypti is unattended by any sterility barriers between populations. This may be because much adaptive plasticity is achieved, without sacrifice of gene flow, by the evolution of balanced polymorphisms.

One of the most remarkable and often quoted cases of interspecific hybridization in mosquitos is that between A. aegypti and the distantly related A. albopictus. MACGILCHRIST (1913) had first noticed copulation in nature between these species, and his surmise that "this coupling seemed unproductive" was confirmed by SIMMONS et al. (1930) in "carefully controlled experiments". Nevertheless TOUMANOFF (1937, 1939), HOANG TICH TRY (1939), DOWNS and BAKER (1949) and KARTMAN (1953) all claimed to have obtained "hybrids" resembling the maternal parent in every detail. Kartman even tested their susceptibility to Dirofilaria immitis. Downs and Baker had suggested that a parthenogenetic mechanism might be involved, despite the contradictory evidence of all reported hybrids (including their own) being of both sexes. TOUMANOFF (1950) not only cited his hybrids as cases of cytoplasmic inheritance, but made the remarkable suggestion

that such a mechanism might be important in promoting the survival of A. aegypti in areas dominated by A. albopictus.

Cytoplasmic inheritance however fails to explain the single paternal-type hybrids claimed by TOUMANOFF (1938) himself and BONNET (1950). Furthermore, an instance involving not a single character but the whole "genotype" would be unique. It could only result from complete suppression of all paternal genes. MATTINGLY (1956) was led to draw a comparison with the plant genus Oenothera. The one really obvious explanation, that these "hybrids" were nothing more than contaminants of one or other parental species, was first suggested by DE BUCK (1942), who failed to obtain any hybrids. This suggestion is hardly considered by MATTINGLY (1956) and is dismissed by KITZMILLER (1953) as unlikely to produce the same result in several different laboratories. To comment on this last statement, there seem to be five possibilities of contamination, viz. :-

(1) loose eggs transferred to or laid in the larval bowl;

(2) some females of the paternal species in the crossing cage;

(3) some males of the maternal species remaining in the crossing cage;

(4) some of the females used were not virgins, because sexes were not isolated until after emergence;

(5) stray males outside the cage copulated through the netting with females inside.

The elementary precaution which one presumes to have been taken in all the experiments was checking that all the males in the cage were one of the two species and all the females of the other. This leaves possibilities (4) and (5) which will always give rise to maternal type offspring. The rearing of such alleged hybrids will then be prone to possibility (1), contamination by stray eggs. These will either be of the paternal species or like the "hybrids" of the maternal species, and so overlooked. Thus, quite contrary to Kitzmiller, contamination alone may be expected to produce just the sort of results obtained.

WOODHILL (1959), taking every precaution to avoid contamination, crossed the species on a massive scale. A single egg out of nearly 50,000 hatched to produce a male, intermediate, in thoracic characters, between A. aegypti and A. albopictus and with the genitalia of albopictus. This is an unequivocal hybrid of the sort normally expected in species crosses and can leave little doubt as to the value of the previous results. Results of other species crosses (McCLELLAND, 1961), here reported, confirms this impression. ROZEBOOM and GILFORD (1954a) record an unsuccessful attempt to cross A. aegypti with A. polynesiensis. LEAHY (1960) has briefly listed 5 possible barriers to interspecific mating.

The hitherto limited study of interspecific and interstrain hybridization in mosquitos has already served a very practical end in defining species, separating disease vectors from similar but relatively harmless forms and clarifying some taxonomic puzzles. It has in addition served to emphasize the diversity of isolating mechanisms that can occur in a single group of insects and hence the danger of extrapolating from few well-worked species.

PHYSIOLOGY AND BEHAVIOUR

Susceptibility to parasites

In a classic series of papers HUFF (1927, 1929c, 1931, 1935) demonstrated variable susceptibility to avian plasmodia of several mosquitos, including A. aegypti. In Culex p. molestus, susceptibility to Plasmodium cathemerium could be altered by selection and was clearly controlled by a single autosomal recessive gene. Heritable susceptibility to other plasmodia has been reported in C. pipiens (MICKS, 1949) and A. aegypti (TRAGER, 1942) but no precise analysis was achieved. Other workers failed to find inherent differences in susceptibility, presumably simply because their material was homogeneous (TATE and VINCENT, 1934; COGGESHALL, 1941; BOYD and RUSSELL, 1943; HOVANITZ, 1947).

ROUBAUD (1937) and KARTMAN (1953) were unable to determine the genetic basis of wide differences in the susceptibility of different strains of A. aegypti to

Dirofilaria immitis. Following similar observations on A. aegypti and Brugia malayi by RAMACHANDRAN et al. (1960), MACDONALD (1961) was able to improve the infection rate in one strain of the mosquito from 31% to 90% in two generations. Subsequently, MACDONALD (private communication) has obtained substantial evidence (seen by the writer) that the single factor involved is sex-linked.

The apparent difference in ability to transmit St.Louis encephalitis virus that exists among strains of C. p. fatigans (HAMMON and REEVES, 1943) suggests a genetic basis for virus susceptibility. This fascinating field does not seem to have attracted subsequent research.

Egg diapause and oviposition

GILLETT (1955b) showed that variation in the depth of egg diapause (GILLETT, 1955a) between two strains of A. aegypti was inherited and correlated with a behaviour difference. Females of the West African strain laid uninterrupted egg batches of strongly diapausing eggs which therefore tended to hatch irregularly, while those of the East African strain laid weakly diapausing eggs in sporadic batches. In both cases some temporal or spatial dispersion of hatching was achieved. Females of one strain mated with males of the other laid eggs of intermediate diapause, showing that this character, unlike exochorionic structure in Anopheles eggs, was dependent on the zygotic genotype.

Females of the same two strains also differed in their ability to produce an ovulation hormone in the absence of the stimulus of mating. Only in the West African strain were eggs laid by virgin females (GILLETT, 1955c). In crosses between the two strains GILLETT (1956) found that the proportion of virgin females laying eggs was intermediate between that of the parents in both the F_1 and F_2. Backcrossing the F_1 to either parent gave proportions of layers intermediate between those of the F_1 and backcross parent. GILLETT (1956) claimed that no elucidation of the genetic mechanism involved was possible beyond a surmise that multiple factors were involved. His data however fit the hypothesis of a single dominant gene, permitting ovulation in virgins, present at a frequency of about 0.45 in the West African strain and 0.015 in the East African strain. Gillett failed to emphasize that the hybrid generations are intermediate in the sense of the frequency of virgin layers, rather than in individual response, differences in the numbers of eggs laid could in any case be controlled by other factors.

WOOD (1961a) showed that two strains in particular, among several of A. aegypti studied, differed significantly in the apportionment of oviposition between the dark and light compartments of a partly divided cage. Females of a DDT-resistant strain (STR) from Trinidad laid 65% of their eggs in the dark side compared with 17% in the case of a DDT-

susceptible strain (AS). The STR strain might be thought to show no real preference, but, since the cage was continuously illuminated and a low proportion normally rested in the dark side, Wood is justified in describing STR as a "dark-laying" strain. The absence of all-or-none responses could reflect different gene frequencies in the two strains or indicate that, under the experimental conditions, the stimuli presented were not discriminatory. Nevertheless, Wood concludes from his failure to select dark- and light-laying substrains, that the STR strain is homogeneous for a "dark-laying tendency". He further assumes, without evidence, that the AS strain is homogeneous for a "light-laying tendency" and that the figures of 65% and 17% are in effect intrinsic values for the two populations homozygous respectively for a factor causing the "dark-laying tendency" and its allele. Expectancies calculated from these assumptions, with some disregard of statistics, give reasonable correspondence with the observed F_1 and F_2 values in crosses between the two strains. The only genetic conclusion justified however is that of heritability, the "light-laying tendency" being dominant over the "dark-laying tendency" with no relation to DDT resistance.

Resistance to insecticides

general

As a physiological response of direct economic significance, resistance in mosquitos to insecticides has

attracted a great deal of attention. The purely toxicological aspect will here be ignored, as will the body of papers that merely report the appearance of resistance in hitherto susceptible populations.

Specific genetic resistance occurs towards three main groups of toxins; DDT and analogues; Dieldrin, analogues and BHC; and organophosphorous compounds (BUSVINE and COKER, 1958), and can be distinguished from generalized resistance or vigour-tolerance (HOSKINS and GORDON, 1956) which, as CROW (1960) points out, is not synonymous with the general fitness that is the "goal" of all natural selection. Vigour-tolerance or multicomponent resistance represents, as the term suggests, increased tolerance to a toxin and is of polygenic origin (SPILLER, 1958 a,b). Specific resistance, on the other hand, confers an ability to detoxify a specific poison, to a physiological level which can be tolerated, and is usually monofactorial. Spiller points out that neither multicomponent nor, except in the case of dieldrin resistance, specific resistant mechanisms, can alone impart the highest degrees of resistance.

There is general agreement that resistance is pre-adaptive in arising through selection of existing genes rather than post-adaptively induced by the toxin (CROW, 1957); the remarkably high frequency of A. gambiae heterozygous for dieldrin resistance in unsprayed areas of Nigeria (ARMSTRONG et al. (1958) supports this. The speed at which resistance

develops has been compared with the almost imperceptible march of natural evolutionary processes by both CROW (1960) and REID (1960). Crow's term "cataclysmic" seems best applied to the sudden onslaught of insecticides, rather than to the rapid development of resistance that results. A parallel example is the development of "industrial melanism" in moths, reviewed by KETTLEWELL (1961).

Even in the absence of any obvious man-made cause, natural selection can be very intense. Summarizing his classic work on an example of such a case involving chromosomal polymorphism in Drosophila, DOBZHANSKY (1961) distinguishes macro-, meso- and micro-evolution. This suggest that the substitution of a resistance gene for its susceptible allele, in a population exposed to insecticides, would be microevolution. The adaptation of the resistant genotype at several other loci, so that it is as fit in the new environment as the susceptible genotype was in the original insecticide-free environment, might then be termed mesoevolution. The rate at which this will happen, let alone macroevolution, will not seem so fast.

dieldrin resistance

Resistance to dieldrin is monofactorial in all species of mosquito so far studied. In A. gambiae DAVIDSON (1956) established dosages which discriminated between homozygous susceptible, heterozygous resistant and homozygous resistant individuals, indicating partial dominance of the factor for dieldrin resistance. Not only was partial dominance of the

resistant gene similarly found in A. quadrimaculatus and A. albimanus, but the discriminating dosages were the same as for A. gambiae (DAVIDSON and JACKSON, 1961a). ROZEBOOM and JOHNSON (1961), who claimed that dieldrin resistance in A. albimanus was fully dominant, failed to use doses high enough to discriminate between heterozygous and homozygous resistants and were working with impure strains. Their conclusion that the F_1 hybrids from susceptible x resistant crosses were more resistant than the resistant parents is probably erroneous, while their use of different techniques is confusing in comparison with Davidson's work. Monofactorial, semi-dominant resistance to dieldrin has also been described in C. p. fatigans (DAVIDSON and JACKSON, 1961a) and A. aegypti (KAHN and BROWN, 1961).

DDT resistance

The level of resistance to DDT shows much wider inter- and intra specific variation than that to dieldrin, indicating the greater effect of genetic background or multicomponent resistance. The mechanism involved is nevertheless demonstrably monofactorial and in Anopheles sundaicus (DAVIDSON, 1957), A. stephensi and A. albimanus (DAVIDSON and JACKSON, 1961a, 1961b) it is recessive, again unlike dieldrin resistance. In A. aegypti, resistance to DDT, while varying from 10 to 1000 times the susceptible level, is always partially dominant (COKER, 1958; QUTUBUDDIN, 1958; KHAN and

BROWN, 1961). Coker's crosses between three resistant strains suggest that either more than one locus is involved or that one of the strains, a Malayan, shows merely vigour tolerance and not true DDT resistance. The resistance spectrum of the Malayan strain supports the former conclusion (BUSVINE and COKER, 1958).

ABEDI and BROWN (1961) demonstrated very beautifully that secretion of larval peritrophic membrane is a mechanism for the physical removal of DDT from the gut in two DDT resistant strains of A. aegypti. After 24 hours exposure to 1 p.p.m. DDT, larvae of a Trinidad strain secreted nearly 9 times as much peritrophic membrane as the susceptible strain. Larvae of the highly resistant Malayan strain, previously investigated (ABEDI and BROWN, 1960, vide infra), on the other hand, secreted little more peritrophic membrane than the susceptible Malayan strain. The LC_{50} of the Trinidad strain used by Abedi and Brown was 1.5 p.p.m., although QUTUBUDDIN (1958) obtained a value of 30 p.p.m. for the same strain.

It is open to question whether this phenomenon of the peritrophic membrane is another genetically distinct type of resistance mechanism. Comparison is made with susceptible strains, having an LC_{50} of 0.08 p.p.m. DDT or less, which are likely to be profoundly affected by a concentration of 1.0 p.p.m. DDT, over 12-fold (in one case 200-fold) the LC_{50}. The hypersecretion of peritrophic membrane may be a normal response to DDT as a gut irritant, which is overridden, in the

case of the DDT susceptible strains, by the toxic action. This hypothesis could be tested by comparing the secretion of peritrophic membrane by both DDT resistant and susceptible strains in response to an irritant of low toxicity.

DDT resistance in C. p. fatigans, though inadequately studied, shows the interesting feature of maternal influence. PAL and SINGH (1958) crossed susceptible with resistant strains and suggested that cytoplasmic inheritance might account for the differences between the reciprocal crosses. In other respects, the very low order DDT resistance involved was inherited as a recessive factor. Another case of slight resistance in this species was studied by ROZEBOOM and HOBBS (1960) who crossed resistant C. p. fatigans from the Philippines with susceptible C. pipiens from America. Unlike Pal and Singh, they found a tendency towards dominance, but again in every case the F_1 from crosses of resistant C. p. fatigans females to C. pipiens males were more resistant than either the resistant C. p. fatigans itself or the F_1 from the reciprocal cross. Furthermore, the F_2 and backcrosses in which the female cytoplasm was derived from C. p. fatigans were more resistant than expected compared with the crosses involving C. pipiens cytoplasm. It is a pity that only 4 out of the 8 possible backcrosses were made, and surprising that the authors neither remark on this obvious maternal effect nor comment on the results of Pal and Singh. It is clear that DDT resistance, in all the species studied, shows a much wider range of variation than does dieldrin resistance.

multiple resistance

Where both DDT and dieldrin resistance occurred together in a single strain of A. albimanus, DAVIDSON and JACKSON (1961a) were able to produce, in addition, strains resistant to either dieldrin or DDT alone and susceptible to the other insecticide. Double resistant strains, and those showing one type of resistance separately, also exist in A. quadrimaculatus A. stephensi and A. pharoensis (DAVIDSON and MASON, in press), but in these cases the single resistant strains were of separate origin and not derived from the doubly resistant strains. The existence of the two types of resistance both separately and together, one partially dominant and the other recessive, evinces their separate genetic identity.

KAHN and BROWN (1961) attempted to separate factors for dieldrin and DDT resistance from a doubly resistant strain of A. aegypti from Puerto Rico (strain PR of the present study), by 4 generations of repeated backcrossing to a susceptible strain with selection for either insecticide separately in different lines. Although in essence the method is sound, its application in this case seems less so. If the factors were linked and homozygous in the strain under test the selection in each generation would remove all the susceptible recombinants. Thus with a recombination value x, a proportion $1 + x^4 - 4x^3 + 6x^2 - 4x$ of the dieldrin (or DDT) genes would remain after 4 generations of selection with DDT (or dieldrin). Instead of attempting to measure such a

reduction, Kahn and Brown looked for a rise in level of DDT (or dieldrin) resistance following DDT (or dieldrin) selection measured by the LC_{50} "derived from dosage-mortality regression lines fitted by eye". To add further to the confusion, they later state that the strain was not pure for resistance. The absence of any significant changes in LC_{50} of either insecticide under the two types of selection led Khan and Brown to the perhaps rather presumptuous conclusion that resistance to DDT and dieldrin is inseparable. They went on to cross the Puerto Rican strain with a susceptible strain homozygous for the recessive genes y and blt, yellow and black tarsi, vide infra. Unmarked F_1 individuals were again crossed to the double-marked susceptible strain giving 50% blt and y offspring. A slight inflexion in the dosage-mortality curve indicated that about 25% of the yellow larvae were heterozygous resistant, from which Kahn and Brown infer 25% crossing-over between the factors for y and DDT-dieldrin resistance. 54% of the F_1 were stated to have been yellow larvae, so that a line homozygous for both resistance and yellow larvae could have been easily isolated, to give much more precise linkage data, by a backcross to the double heterozygote.

selection and population studies

In most of the work reviewed above the measure of resistance has been the LC_{50}, or median lethal concentration, determined from the regression line of probit mortality

plotted against serial concentrations of insecticide. DAVIDSON (1958 a,b) has drawn attention to the shortcomings of this method which fails to distinguish individuals and advocated the use of a discriminating dosage to separate resistant from susceptible genotypes. It seems doubtful, however, whether there is any way of assessing the potentiality of a natural population to develop resistance, particularly when recessive as in the case of DDT.

MACDONALD (1959), applying principles of population genetics, has calculated that only 14 generations with a reasonable selection coefficient of 0.9 are necessary to raise the frequency of a recessive resistance gene from 0.01 to 0.97. Such a none too rare initial gene frequency represents one recessive homozygote in 10,000 mosquitos. The establishment of even such a minimal limit with 95% confidence would require testing the impracticably large sample of 9,500 mosquitos with the discriminating dose. Seen in this light, negative findings of DDT resistance in natural populations are of limited value.

Even in laboratory populations, failure to detect a low initial frequency of resistant phenotypes, followed by a 500-fold increase in resistance in 7 generations of selection, led ABEDI and BROWN (1960) to suspect a "post-adaptive Lamarckian phenomenon" in a Malayan strain of A. aegypti. The course of selection in this case was further obscured by the fitting of straight probit-mortality regression lines to data which did

not extend to complete mortality. Rapid reversion following relaxation of selection pressure in the earlier generations indicated that resistance was associated with lower fitness.

Other attempts to select resistant strains in the laboratory have met with varied results. KUHLOW (1957) failed to increase by any significant amount the degree of resistance of A. stephensi and A. atroparvus to either dieldrin or DDT. DAVIDSON (1958a) records a similar failure to increase DDT-resistance in A. gambiae by selection with either DDT or BHC. His results of DDT selection on A. stephensi giving a 6-fold increase in resistance, and similar low-order responses to DDT by A. aegypti (SHIDRAWI, 1957; SURTEES, 1958) are probably merely improvements in tolerance. In contrast, high specific resistance to DDT in response to selection has been reported by MOSNA et al.(1959) working with A. atroparvus, BURNETT and ASH (1961) with A. pseudoscutellaris, and in the case of A. aegypti above (ABEDI and BROWN, 1960).

The explanation of the diverse results of selection is simply, as DAVIDSON and MASON (in press) point out, that selection in a relatively small laboratory population will rapidly increase the frequency of specific oligogenes for resistance should these be present. It may be added that the restricted origin of most laboratory populations constitutes a genetic bottleneck, reducing variance and increasing minimum gene frequencies, so that genes for resistance, if present at

all, will usually be so at frequencies permitting rapid selection.

cytogenetics and resistance

An as yet little understood effect associated with resistance to either DDT or dieldrin in Anophelines is an increase in heterozygous and homozygous chromosome inversions, first observed by HOLSTEIN (1957) in resistant and susceptible strains of A. gambiae kept under the same conditions in London. This was confirmed in A. atroparvus by FRIZZI et al. (1957) and D'ALESSANDRO et al. (1957, 1958). FRIZZI and HOLSTEIN (1956) noticed that a higher larval rearing temperature had the same effect. D'ALESSANDRO et al. (1961) confirmed this and also showed that the increase in temperature alone also led to increased DDT tolerance. They concluded that such tolerance was heterotic and the result, not the cause, of chromosomal heterozygosity.

The evident lack of coherence in studies of insecticide resistance in mosquitos no doubt reflects the economic and political impetus attaching to eradication programmes. Techniques and approaches have been standardized before the underlying problems have been fully appraised. There is an urgent need for more rethinking along the lines of basic population genetics.

FORMAL GENETICS

General

early work and studies on larval pigmentation

HUFF (1929 a) was the first to demonstrate an example of Mendelian inheritance in mosquitos. Green colour in the larval fat bodies of C. p. molestus was clearly controlled by a single gene recessive to that producing normal red-brown pigment. Larval colour, so easily observed, has been the object of later studies.

GHELELOVITCH (1950) found that a gray-green colour in the same species, C. p. molestus, was monofactorial, but dominant over the normal yellow, and so was presumably not the same gene as that causing greenness in Huff's material. SPIELMAN (1957) crossing anautogenous and autogenous strains of C. pipiens, showed that a yellow colour was recessive to pink, and autosomal.

While it is obvious that different genes have been studied in these three cases, LAVEN (1957 a), who was able to duplicate Huff's work, surmised that they belonged to an allelic series. The opposite opinion, that more than one locus is involved was favoured by CRAIG and GILLHAM (1959) which, in the absence of direct evidence, seems rather presumptuous in view of their own findings in A. aegypti. In this species Craig and Gillham concluded that larval colour is probably controlled by a series of alleles at a single autosomal locus.

A yellow larval variant $\frac{y}{y}$, lacking pigment granules in the fat body is clearly recessive to the gray wild-type $\frac{y^+}{y^+}$. Other colour forms such as brown y^b, seem likely to be allelic. A possible fourth allele y^m was responsible for a semi-lethal melanotic condition. Melanotic x yellow larvae were wild-type which does not rule out allelism since it is likely that the effects would be additive.

A similar, though more lethal, melanotic mutant which never survives to an adult has been isolated in C. p. fatigans by KITZMILLER (1953). On the basis of observed and expected ratios in the progeny of presumed heterozygous single-pair matings, Kitzmiller concluded that the condition was the expression of a triple recessive. Unfortunately, such calculations make the assumption difficult to confirm, that each gene is fully penetrant. LAVEN (1957a) isolated a similar lethal melanotic larval mutant mel, which behaved clearly as a fully penetrant autosomal recessive. Using paper chromatography, LAVEN and CHEN (1956) showed reduced amounts of several amino acids in these melanotic larvae compared with the normal form.

The only genetic study on larval Anophelines so far published concerns colour pattern in A. quadrimaculatus. While definitely heritable, COGGESHALL (1941) was not able to demonstrate a monofactorial basis. There is nevertheless an indication by DAVIDSON and MASON (in press) that further work is presently in progress.

Further to his study of larval colour, GHELELOVITCH (1950) concluded that a spotted pattern on the adult abdominal sternites of C. p. molestus was polygenically controlled. Although some of the matings were infertile (vide supra), ROUBAUD (1945) and ROUBAUD and GHELELOVITCH (1950) in earlier crosses had found that the spots were semi-dominant.

sex determination

Until GILCHRIST and HALDANE (1946, 1947) found that a spontaneous recessive white-eyed mutant of C. p. molestus was partially sex-linked, nothing at all had been known of the mechanism of sex determination in mosquitos. Their data showed that there were two types of males heterozygous for white-eye depending which parent had been white-eyed. When those with white-eyed mothers were mated to heterozygous or homozygous white-eyed females, almost all the expected proportion of white-eyed progeny were female. When heterozygous males with white-eyed fathers were similarly mated, almost all the segregating white-eyed progeny were males. Gilchrist and Haldane concluded that sex was determined at a single locus, the factor for maleness M being dominant, so that the males were always heterozygous and heterogametic. The recessive gene for white-eye was linked to the sex-locus with crossing-over, unlike Drosophila, in both males and females, at a frequency of 6.26%. Another partially sex-linked gene causing some wing-vein fusion shows 1.74% crossing-over with the sex-locus (LAVEN, 1957a). In

Anopheles there are no reports of sex-linked genes, though some have been very recently isolated (MASON, personal communication), and evidence for crossing-over in males has only been inferred cytologically (FRENCH and KITZMILLER, 1961).

KITZMILLER and LAVEN (1958) used marker genes to test for multiple fertilization in C. p. fatigans and C. p. molestus. They concluded that a single female cannot produce in the same egg raft, eggs fertilized by more than one male. VANDEHEY and CRAIG (1958) used the yellow larval mutant to show that females of A. aegypti can in contrast lay such mixed egg batches.

gynandromorphs and intersexes

Despite long ignorance of the mode of sex-determination in mosquitos, reports of gynandromorphs are by no means rare, these are listed in the final Table, XXIX. Nevertheless, gynandromorphs as such are far from common, considering the few that would escape detection by any but the untrained eye. According to WARREN and HILL (1947) 5 gynandromorphs were recorded out of more than 2 million field-caught mosquitos examined over some years in their laboratory. What is remarkable is the complete absence of gynandromorphs or sexual mosaics, having some parts of the body pure female and others pure male. These must be distinguished from intersexes in which all parts of the individual are intermediate between

male and female (SINNOTT et al. 1958). Cases of intersexes in mosquitos are rarer, but BLASQUEZ and MAIER (1951) clearly demonstrated heritable intersexuality in a colony of C. p. fatigans, by obtaining a total of 50 "gynandromorphs" in 5 consecutive generations under DDT selection. What was presumably the homologue in C. p. molestus was shown by LAVEN (1955a), using marker genes, to be caused by an autosomal recessive. Disturbance of the sex ratio indicated that the intersex was a modified male. In Aedes stimulans female characters may be induced in genetically-intended males by rearing the larvae at temperatures higher than those naturally encountered (HORSFALL and ANDERSON, 1961).

The occurrence of three gynandromorphs proper of Aedes punctor close together in a single locality (EDWARDS, 1917) and three of C. p. molestus in a single laboratory strain (GILCHRIST and HALDANE, 1947) argues strongly in favour of a heritable mechanism. LAVEN (1957a) established a gynandromorph-producing strain and showed that a single recessive factor was responsible. Using a dominant marker-gene he further demonstrated that a gynandromorph showing the mutant effect unilaterally, must have arisen from at least two sperm, one carrying the dominant mutant and male determining, the other the recessive allele and female determining. In Laven's 57 gynandromorphs the male-female axis was quite arbitrarily placed. A similar conclusion is reached by VANDEHEY and CRAIG (1961) who, in an an abstract, record 80

gynandromorphs of A. aegypti during a 3-year period. They note that bilateral and anteroposterior gynandromorphs are equally frequent but that mosaics (= intersexes ?) are rare. This is also evident from the totals of the other entries in Table XXIX.

search for natural and radiation-induced mutants

The white-eyed mutant of Gilchrist and Haldane occurred spontaneously in a long-established laboratory strain, but KITZMILLER (1958) comments that such natural mutants seem rare in mosquitos. Certainly he (KITZMILLER, 1952) had sib-mated single pairs of C. p. fatigans for 20 generations, and C. pipiens, for 12, without finding any mutants. SMITH-WHITE and WOODHILL (1954) failed to observe any mutants by inbreeding A. s. scutellaris. By contrast, LAVEN (1955b, 1956c, 1957a, 1958) isolated more than 12 visible and heritable variations in C. p. molestus, mostly following X-irradiation, but at least four occurred spontaneously. It is particularly interesting that KITZMILLER (1958) who subsequently treated C. p. fatigans with X-rays obtained, inter alia, several variants closely parallelling some of Laven's mutations in C. p. molestus. In view of the genetic compatibility between the two species it seems probable that these are true homologues. A spread-wing variant was a possible mutant isolated by PAL and KRISHNAMURTHY (1959) following X-irradiation of C. p. fatigans. The only mutant isolated in A. gambiae, after a low dosage of X-rays, by

JACKSON (1957), was similar to a variant occurring naturally elsewhere. The main effects of gamma radiation on C. p. fatigans and A. aegypti, studied by GHOSH et al. (1961 a,b) and HATI and GHOSH (1962) were developmental and physiological; no mutants were reported. VANDEHEY and CRAIG (1962) irradiated A. aegypti with both gamma and X-rays but concluded that most of the mutations causing structural modifications subsequently isolated were present before treatment, as were 9 colour mutants (CRAIG and VANDEHEY, 1962).

Although BATEMAN (1955) pointed out that a comparison of the mutagenic effect of ingested P^{32} and X-rays on Drosophila was hardly valid, it remains that a much higher number of visible mutations resulted from long-term exposure to P^{32} compared with short-term X-irradiation (BATEMAN and SINCLAIR, 1950). The effect of P^{32} (as Na_2PO_4) and also Sr^{89} (as $SrCl_2$) on larvae of A. aegypti was investigated by BUGHER and TAYLOR (1949) and HASSETT and JENKINS (1949) in connection with radioactive marking. ABDEL-MALEK (1961), similarly investigating the use of P^{32} on C. p. molestus, determined the lethal dose and noted the retarding effect of lower doses, but neither worker observed (or looked for) any mutagenic effects. P^{32} may possibly hold great promise in this field.

Aedes aegypti

natural variation in colour

Unlike Culex and most other well studied species, the

variation in colour of A. aegypti is legend. This is reflected to some degree by the plethora of synonyms for the species, and is partly due, almost certainly, to the normally distinctive colour pattern in which small variations are easily noticed. ROBINEAU-DESVOIDY (1827) and F.H.TAYLOR (1914) described forms lacking the typical lyre-pattern on the mesonotum; THEOBALD (1901) described a pale variety queenslandensis from Northern Australia and one with black-tipped metatarsi luciensis from the Guianas. EDWARDS (1941) described a variety with more extreme tarsal darkening as atritarsus.

The first breeding experiments with colour forms were reported by HILL (1921) in Queensland, Australia, who observed that those A. aegypti breeding in scrub were darker than those occurring in the vicinity of houses. Hill found that, while the paler form bred true, the dark produced in each generation after the first both pale, dark and intermediate forms.

CONNAL (1926, 1927) analysed the colour variation of the metatarsi and abdomen of 1000 males and 1000 females. The abdomen varied from being completely black, with the exception of the lateral spots, to completely covered with pure white scales. Intermediate forms were speckled or brindled with white scales. The lateral spots varied in size and were occasionally purple (sic). Either, both or neither basal and apical pale tergal bands were present. The metatarsi showed great variation in the width of the basal pale rings ranging

from the normal through var. luciensis to var. atritarsus. The only constant feature seemed to be the anterolateral spots though even here the centre lines varied in length, breadth and colour. There was clearly no correlation between the metatarsal variants and any of the abdominal variants.

In 3 out of 4 single-pair crosses of white-abdomen females to various males and in 1 F_1 mating Connal obtained clear segregation, indicating that either or both parents were heterozygous, the fourth cross and another 2 F_1 matings were more homogeneous. Connal however merely seeks parental resemblances and makes no attempt to explain her results on Mendelian lines, although this is hardly possible with only two families exceeding 7 offspring.

BONNE-WEPSTER and BRUG (1932) reported very considerable variation in A. aegypti in Indonesia, much similar to that described by Connal, including forms lacking the pale basal bands. Of the tarsal variants only luciensis was reported. No crosses were attempted. FLOCH et al. (1942), in material from Cayenne, illustrate not only var. luciensis and wild-type but 3 intergrades as well, and also the clearly oblique junction of the black-and-white bands. Some simple crosses indicated that wild type x wild type gave at most progeny with just a few black scales, no complete rings. Matings between fully expressed var. luciensis, however, yielded some wild-type in the F_1, but fewer than 3% after two subsequent selective generations. It is hard to avoid a conclusion of

polygenic control. SHIDRAWI (1955), in a brief report, unfortunately published no data to support his assertion that there was a tendency towards inheritance of maternal scale colour in reciprocal crosses between dark and pale A. aegypti.

colour in relation to bionomics

In the coastal, or near coastal, regions of Kenya and Tanganyika, the frequency of pale forms of A. aegypti has been shown to be clearly higher in populations in and around human dwellings than in those of the nearby bush or plantations (TEESDALE, 1955; VAN SOMEREN et al., 1955, 1958; McCLELLAND, 1960b). Although MATTINGLY (1956) pointed out that these are similar observations to those of HILL (1921, vide supra), there is the important distinction, clarified below, that the dark forms involved are rather different. The implied correlation between colour and behaviour differences, or the possible association of paleness with a cryptozoic habit (MATTINGLY, 1956), were among the many problems which called for a reappraisal of the whole biology and taxonomy of A. aegypti. In doing this, MATTINGLY (1957, 1958) began with an attempt to define, taxonomically, the bewildering range of colour variation.

MATTINGLY (1957) recognized as a subspecies, A. aegypti ssp. formosus (Walker), a form restricted to sub-Saharan Africa "where it is the only form known to occur, except in coastal districts and in one or two areas of limited inland

penetration". As well as the dark-scaled areas being generally blacker, this form "never has any pale scales on the first abdominal tergite".

The type form (accepted in the present study as the wild-type of genetic parlance) is defined somewhat loosely by MATTINGLY (1957) to include forms distinctly paler than ssp. formosus but in which "extensions of pale scaling, if any, are limited either to bleaching of the two dark areas on the back of the head, or to the presence of pale scaling on the first abdominal tergite, or to both in combination". This leaves the third form to include all individuals in which the extent of pale scaling exceeds the above limits. This last assemblage, covering a huge range of colour from those almost referable to type form to those with the abdomen and mesonotum completely bleached, is taken as var. queenslandensis.

recent developments in formal genetics

CRAIG (1958) briefly reported 5 spontaneous mutants in A. aegypti affecting scale colour of palps, thorax, abdomen and legs, and suggested their usefulness as genetic markers. Of these s was allelic (CRAIG and VANDEHEY, 1962) with the recessive gene causing replacement of the normal silver lateral abdominal spots by dull white scales, which McCLELLAND (1960b) later isolated as "white-spot". McClelland was able to analyse the seemingly continuous variation in abdominal colour by breaking it into 11 generalized colour grades, and

concluded that genetic control was multifactorial. CRAIG and VANDEHEY (1962), on the other hand, attributed the control of abdominal colour to a single semi-dominant factor and claimed that McClelland's figures were consistent with this hypothesis. They pointed out, however, that different strains were involved. McClelland also showed that the pale form, largely restricted to houses, seemed to have a slower rate of larval development than either the dark form or the hybrid between the two. In contrast to the dark form, the pale form readily accepted guinea-pig as a source of blood after 3 or 4 generations feeding exclusively on man. Both these findings could provide a clue to the association of the pale form with houses.

CRAIG et al. (1961) listed 41 mutants of A. aegypti which were definitely heritable and a further 41 possible mutants about which little was known; homology with some Culex mutants (vide supra) is indicated. Craig et al. analysed 25 strains for the recessive y gene (vide supra) affecting larval pigmentation. Although the frequency of y homozygotes in one particular strain remained very similar throughout 13 generations of intense selection with DDT, they point out "different selective pressures acting in various laboratories resulting in changes in the frequency of the y gene". Nevertheless, the frequency of the y gene is calculated "by the Hardy-Weinberg Law", despite the clear failure of the y gene to satisfy the conditions under which the law is valid.

CRAIG et al. (1961) also analysed 37 single pairs from 4 strains for hidden recessives and concluded that the natural frequency of mutants exceeded that of most Drosophila populations and was 1.5 times higher than that in C. pipiens following irradiation. They point out that the relative lack of morphological differences in Anopheles and Culex, accompanied by sterility barriers, contrasts with the high morphological (colour pattern) variance and absence of such barriers in A. aegypti. Here again is probably a confusion of cause and effect - might it not be the absence of noticeable colour differences, due to the lack of any sharply defined pattern, that has obscured biologically distinct species in Anopheles and Culex ? For example, the analogue of the sibling species in Anophelines may be the variety of Stegomyia species in E. Africa. Groups of these differ by little more than scale colour patterns.

CRAIG et al. (1960, 1961) described in A. aegypti a factor, inherited through the male, causing a greatly enhanced ratio of males to females. Strains carrying it give single-pair progenies varying from 0% to 51% female with a mean near 22%. It is difficult to agree with the authors that such a "male-producing factor" could be useful in control operations, unless penetrance was such as to give consistently very few females. Even then, the increased chances of any female being fertilized might offset their lower numbers. It is clear however that the sex ratio in A. aegypti is unusually

variable and that little significance can be attached to other reports of wide departures from a 1 : 1 ratio, for, although both McCLELLAND (1960 b) and (in a longer discussion) WOOD (1961 b) agreed that the effects were inherited paternally, they were not aware of Craig's work.

In two recent papers, CRAIG and VANDEHEY (1962) and VANDEHEY and CRAIG (1962) describe in greater detail 9 mutants affecting colour pattern and 30 causing structural modifications in A. aegypti. Only 6 of these are placed in any of the three linkage groups corresponding to the 3 chromosomes. Group I is defined as that containing the sex factor and Group II that containing y (CRAIG and GILLHAM, 1959). Of the 6, recombination data is only given for 3 in Group II, all with respect to y. The details of this work will be referred to later.

THE FUTURE IN MOSQUITO GENETICS

The significant details of most of the past work on the genetics of mosquitos have been briefly sketched from its early naive and haphazard beginnings to its present status as a distinct and increasingly important department of mosquito studies. Future developments must be seen in relation to the special advantages offered by each of the three groups mainly involved. The Anophelines, of which several species are favoured, are pre-eminently valuable as cytogenetic material, C. p. molestus in particular among its genus combines the advantage of extreme stenogamy with needing no specially heated insectaries and shows the interesting phenomenon of cytoplasmic inheritance. A. aegypti in particular among its genus, on the one hand, is probably the most highly studied of any single mosquito species (see CHRISTOPHERS, 1960), and on the other probably has on balance the most favourable combination of ease of culture, drought-resistant eggs and high variability (MATTINGLY, 1956; CRAIG et al., 1961). If the promise of an adequate cytogenetic technique in this species is fulfilled (MESCHER, 1960; CRAIG et al., 1961), it could become one of the most useful of experimental insects in genetics.

The large body of literature available on the responses of mosquitos, and A. aegypti in particular, to various stimuli would make them particularly good objects for developing the field of behaviour genetics, advances in which would probably

supply a powerful weapon in the fight to control or eradicate disease vectors. Before this is feasible, it is essential to have a sound formal genetics as a base. This has been the paramount object of the present study, although it was originally inspired by the writer's personal experience of the great diversity of colour and biting-behaviour in A. aegypti in East Africa (McCLELLAND, 1959, 1960a). This prompted the preliminary study (McCLELLAND, 1960b) discussed above. It is a happy coincidence that some of the most useful genes in linkage studies are those causing the colour differences which are so perplexing to the field worker.

PART II

MATERIALS AND METHODS

MATERIALS AND METHODS

LIVING MATERIAL

The present work was based entirely on living material. Initially, exploratory study was confined to a strain DH of *Aedes aegypti* (L.) from New Delhi derived via the National Institute for Medical Research, Mill Hill, through the kindness of Dr. F. Hawking (later batches were received direct from The Malaria Institute of India), followed by strain GA kindly obtained by members of the Kenya Medical Department from the locality personally studied by the writer in 1957-58. Strain JA, of Indonesian origin, was then obtained from Mevr. J. Bonne-Wepster of Amsterdam where it had been kept for some years. Liaison was then established with Dr. G. B. Craig of Notre Dame, Indiana, whose "mosquito genetics project" involves the maintenance of many strains of *A. aegypti* of diverse origin, some of which were gratefully received.

At this stage it was decided to obtain strains, wherever possible, direct from the country of origin. Detailed requests were mailed to nearly 100 workers in nearly all countries where *A. aegypti* occurred. The response was gratifying, over 50 separate consignments were received, not

all of which were viable. Table I lists all the viable strains received which were examined. An additional African strain, EN, colonized for about 25 years at the London School of Hygiene and Tropical Medicine, was used but not maintained by the writer. Each geographical strain is referred to by the two code letters only. Most material was field-collected as larvae; eggs obtained from the first laboratory generation being mailed to London. These are described under "history" as "W(F_1)". In some instances more than one laboratory generation was required to produce eggs suitable for despatch "W(F_2)". In other cases eggs laid directly by wild-caught females were sent "W(P)". The eggs from Ganda and Rabai, Kenya, were laid on strips of paper lining receptacles (bamboo pots, tins, etc.) sited in the natural habitat. These eggs thus conformed closely to a natural sample, but mortality was unfortunately high. Colonies of all the strains listed were run for several generations at least, but less interesting material was discarded periodically to make room for newer strains and mutant strains. The total number of colonies was limited for practical reasons to about 35.

THE LABORATORY ENVIRONMENT

The insectary consisted of three rooms interconnected in a row and heated by thermostatically-controlled electric convectors. An extractor fan in the third room drew an air current from an unheated vestibule into the first room, from the first room to the second and from the second to the third.

TABLE I

CODE	PLACE and COUNTRY of ORIGIN	HISTORY	SENDER	DATE
AO	? Algeria via Germany	(Craig)	G. B. Craig	1/60
BK	Bangkok, Thailand	P W(F_1)	C. Yamarat	5/61
BLP	(marker strain)	(Craig)	G. B. Craig	10/60
BLTS	(marker strain)	(Craig)	G. B. Craig	7/60
CC	Cúcuta, Colombia	(Craig)	G. B. Craig	3/60
CN	Colombo, Ceylon	P W(F_1)	W. A. Samarawickrema	6/61
CR	Carriacou Island, Grenadines	P C(½)	F. R. S. Kellett	6/61
CT	Calcutta, India	P W(F_1)	S. M. Ghosh	7/61
DC	Dacca, E. Pakistan	P C(½)	N. A. Kuraishy	3/62
DH	New Delhi, India	P C(≈5)	Malaria Inst. of India	1/60
DK	Thies nr. Dakar, Senegal	P W(F_1)	M.Kramer via E. Abonnenc	1/61
EK	Ernakulam, Kerala, India	P W(F_2?)	Malaria Inst. of India	7/61
EO	El Obeid, Kordofan, Sudan	P C(2)	M. Qutubuddin	3/60
FS	Karankasso, Upper Volta	P C(?)	J. Hamon via R. J. Wood	10/59
GA	Ganda, nr. Malindi, Kenya	P&F W(P)	G. Oketch via M. Furlong	many
GH	Salakope, Ghana	P W(F_2)	H. vanderkaay via Craig	8/61
HW	Milolii, Kona, Hawaii, USA	P W(F_1)	P. Y. Nakagawa	6/61
JA	Djakarta, Indonesia ■	P C(6)	J. Bonne-Wepster	1/60
JD	Jeddah, Saudi Arabia	P W(F_1)	S. Afifi	2/61
JM	Jamaica	P C(½)	P. Rice	1/62
KD	Kaduna, Northern Nigeria	P C(?2)	M. W. Service	3/61
KN	Karen, nr. Nairobi, Kenya	F W(P)	E. C. C. van Someren	5/61
KR	Karachi, W. Pakistan	P W(F_1)	S. Ashrafi	10/61

....continued on following page

TABLE I continued

CODE	PLACE and COUNTRY of ORIGIN	HISTORY	SENDER	DATE
KW	Key West, Florida, USA	(Craig)	G. B. Craig	3/60
MA	Port Swettenham, Malaya	P W(F_1)	R. H. Wharton	10/60
MB	Miami Beach, Florida, USA	P W(F_1)	J. Porter	4/61
MI	Mornington I., N. Australia	P C(?)	A. R. Woodhill	8/60
MM	Miami, Florida, USA	F?W(F_1)	J. Porter	1/62
MY	Mandalay, Burmah	P W(F_1)	M. Tu	2/62
NJ	Djakarta, Indonesia	P C(1)	R. Harris	6/60
NR	Nairobi, Kenya	P W(F_2)	E. C. C. van Someren	6/60
PH	Manila, Philippines	P C(?)	F. E. Baisas	1/61
PN	Pensacola, Florida, USA	P W(F_3)	H. B. Morlan	4/61
PR	Isla Verde, Puerto Rico	(Craig)	G. B. Craig	5/60
PS	Makun, Pescadores Is.	P C(3)	J. C. Lien	5/61
RB	Rabai, nr. Mombasa, Kenya	P&FW(P)	M. Furlong	7/61
SA	Durban, Natal, S. Africa	P C(20)	J. Muspratt	7/60
SG	Singapore	P W(P)	A. Rudnick	1/61
SK	Salakope, Ghana	P W(P)	W. Z. Coker	10/61
SN	Paramaribo, Surinam	P W(F_1)	E. van der Kuyp	2/61
SO	Sokode Etoe, Ghana	F W(F_1)	W. Z. Coker	10/61
SV	Suva, Fiji Isles	P W(F_1)	Director of Med. Services	4/61
TA	Morogoro, Tanganyika	F W(P)	D. F. Clyde	10/60
TN	nr. Kauriro, Tana R., Kenya	P W(F_2)	D. M. Minter	5/62
TR	Trinidad	(Craig)	G. B. Craig via R. J. Wood	10/59
TV	Townsville, Qu., Australia	P W(3)	M. F. Day via D.H.Colless	10/60
TW	Kaohsiung City, Formosa	P C(3)	J. C. Lien	5/61

....continued on following page

TABLE I concluded.

CODE	PLACE and COUNTRY of ORIGIN	HISTORY	SENDER	DATE
VL	Vellore, Madras, India	P $W(F_2)$	R. Reuben via H. Trapido	3/61
VZ	Barquisimeto, Venezuela	P $W(F_1)$	M. Dorante	7/61
WL	Waltair, Andrah, India	P $W(F_1)$	P. N. Ganapati	4/61
YD	Yaoundé, Cameroun	P $W(F_1)$	H. Bailly-Choumara	10/61

OTHER SPECIES OF STEGOMYIA

SPECIES	PLACE and COUNTRY of ORIGIN	SENDER	DATE
albopictus	Madagascar	A. Grjebine	1/61
apicoargenteus	Entebbe, Uganda	J. D. Gillett	5/61
deboeri	Karen, nr. Nairobi, Kenya	E. C. C. van Someren	5/61
mascarensis	Dauguet Forest, Mauritius	R. Mamet	4/61
metallicus	Ganda, nr. Malindi, Kenya	G. Oketch	7/61
simpsoni	Ganda, nr. Malindi, Kenya	G. Oketch	10/60
woodi	Ganda, nr. Malindi, Kenya	G. Oketch	9/61

TABLE I. Details concerning the mosquito strains used.

NOTES-Under HISTORY (Craig) - details in CRAIG et al. (1961)

P = peridomestic or domestic habitat

F = feral or "bush" habitat

C = laboratory colony, number of years colonized in parenthesis

W = recent field collection, the generation received in parenthesis (P) (F_1)...etc.

DATE The month and year in which received for the present study

* this colony was maintained in Amsterdam, Holland.

The first room was therefore only coarsely temperature controlled, used for rearing larvae only and not humidified. The humidity in the second and third rooms was however maintained at 70 ± 5% relative humidity. This was achieved by means of a humidostat controlling a simple electric boiler in each room. The periodically generated steam was circulated by small fans. The second or middle room was used for most of the genetic work which mainly involved single pair matings. The third room contained the colony cages and egg store.

Temperature, as recorded by thermograph in any one part of the three rooms, was constantly to within ± 0.5°C (slightly wider limits in the first room), but lack of adequate heat insulation and air circulation led to variation from the mean of up to ± 1.5°C on the horizontal plane of the working benches and a much greater vertical variation. Differentials were greater in winter due to the colder walls. It is difficult therefore to specify the mean temperature. The temperature of water in the larval bowls was usually close to 26.5°C, that of the air near the colony cages about 28°C. Examination of adults and hand-mating were however carried out at normal room temperature of about 18°C.

General illumination consisted of an 80w fluorescent tube in each room time-switch controlled to a 12/24-hour cycle. The third room had in addition 4 100w tungsten lamps time-switch controlled to come on one hour after the general

lights and extinguish one hour before the general lights, giving a simulated sunshine period. The first and second room had extra lights switched on during working periods, and the first room received some natural daylight.

GENERAL METHODS

HATCHING OF EGGS

Eggs received from abroad and those laid in the laboratory were hatched by submergence in water that had been deoxygenated by cooling a sealed flask of boiling water. Where few or none of the eggs hatched, they were always removed from the water, dried and resubmerged in freshly deoxygenated water before being discarded as inviable.

REARING OF LARVAE

After hatching, larvae were transferred to numbered enamel bowls containing about 550 ml. tapwater that had been allowed to equilibrate to room temperature. A "Perspex" cover prevented contamination by foreign eggs, dust, etc., reduced evaporation (and consequent temperature differential) and prevented escape of any prematurely emerging adults. A label attached to each cover bore family data, bowl number, wool colour-code (vide infra) and date of hatching (Plate IIa).

Liver powder (Nutritional Biochemicals Inc.) initially proved an excellent scum-free larval food when added daily in small amounts; a soluble fraction induced a bacterial flora

suitable for the smallest larvae while the insoluble particles were ingested by later-stage larvae. Subsequent batches of the same product however were entirely soluble and quite unsatisfactory because a thick scum formed, lethal to young larvae, while the absence of a particulate fraction delayed the nutrition of older larvae. A modified food was therefore used; this was prepared by mixing roughly equal amounts of the soluble liver powder and natural wheat germ ("Froment") with enough water to form a thick paste. This was vacuum-dried and granulated. The granules slowly disintegrated in water releasing food particles and scum-forming matter at a rate comparable to the food intake of the larvae, so that the water tended to remain clear. Nevertheless, the water in the larval bowls was usually changed once during each rearing.

HANDLING OF PUPAE

Pupae of colony material were simply placed in small beakers of clean tapwater inside the cages. The genetic work however demanded some degree of isolation of pupae to prevent damage, to facilitate handling and to ensure that most of the females were virgin. A "dry" method of handling pupae was accordingly modified from an adaptation by ROSS and GILLETT (1950) of the technique first described by BATES and ROCA-GARCIA (1945). The rolling-up and tamping down of lengths of absorbent paper into the standard 25 x 65 mm glass shell vials, although extremely time-consuming, was suggested by Gillett because the cotton wool pads used by Bates and

Roca-Garcia had to be covered by filter paper discs to prevent entanglement of the mosquitos by loose cotton.

In the present work, in order to prepare smooth pads quickly, a principle of paper making was applied. A hollow brass plunger was turned to fit the shell vial (Plate II b). The base of the plunger was a perforated plate (2), the narrower top piece was connected to a water-operated vacuum pump. A bypass-hole in the side of the top piece (1) could be closed by being covered with a finger. Operation was as follows: Shell vials were processed in wire racks of 40 at a time. The vials were half-filled with water, for speed, by almost vertical immersion of the whole rack in water. A lump of absorbent white cotton wool was pushed into each vial. With the water vacuum pump operating and the bypass-hole closed, the plunger was then used to tamp the cotton wool and suck out the water simultaneously. When air was heard entering the plunger the bypass-hole was uncovered, to release the suction, and the plunger withdrawn. With such a method a rack of vials could be given pads in 5 minutes.

For handling pupae a simple pipette was used. This was made from a straight 15 cm length of glass tube, internal diameter about 5 mm and fitted with a rubber teat. Male and female pupae, sexed by their size, were pipetted separately from the larval bowls, washed in tapwater, and transferred in groups of up to 4 on to the cotton wool pads of the vials. The excess water was withdrawn and each vial closed with non-

absorbent coloured cotton wool. Ten different wool colours were available, giving potentially 55 different one- or two-colour codes. Each larval bowl (or family if this was in more than one bowl) had an individual colour code which was not repeated within a two-week period, thereby saving individual vial labels. The racks, containing up to 160 pupae each (Plate III c) could be conveniently stacked one upon another.

HANDLING OF ADULTS

Sucking tube

A venturi-type vacuum pump designed for water operation was attached to a compressed air supply and functioned efficiently at a pressure of about 2 atmos. Obstruction of a tube connected to the outflow at once converted "suck" to "blow". This was useful in clearing dust and debris, etc. collecting in the variety of apparatus (described in appropriate sections below) which was connected to this pump by flexible rubber tubing. The sucking tube consisted essentially of a 25 cm "Pyrex" glass tube, internal diameter about 1 cm tapered apically to 5 mm. Across the tube, in an annular groove about 20 cm from the narrow apex, was fitted a disc of fine woven stainless-steel mesh. With the air suction operating, adult mosquitos could be speedily drawn into the sucking tube out of the pupal vials or cages. Similar sucking tubes connected by a length of rubber pipe to a mouth-

piece were used for other routine adult handling.

Etherizer

A 500-ml narrow beaker partially filled with ether was closed with a cork bung through which passed two tubes. One tube reached almost to the bottom of the beaker, so that air drawn down it bubbled through the ether. The other tube only just projected below the cork, and above was bent horizontally and widened into an orifice to receive and fit the tapered end of the sucking tube. When the latter was held in the orifice, ether-saturated air was drawn over the mosquitos. Then the vacuum line was pinched and the sucking tube removed from the orifice, so that the anaesthetized mosquitos could be shaken or blown out into a small lint-lined dish (Plate IV e^{3}).

Examination and recording

Mosquitos were handled with watchmakers' forceps on a plasticine surface (Plate IV e^{3}), and viewed individually under high illumination at x20 to x60 magnification using a "Baker" stereo-microscope on an extension stand. A large "Perspex" stage with hand-rests was constructed (Plate IV e^{4}) and a makeshift foot-operated focussing control (Plate IV e^{2}) fitted. Any pecularities were recorded and a mark scored under an appropriate heading on a proforma. Each mosquito selected for mating was returned to an individual vial which was given a serially numbered adhesive tape label bearing details of sex, phenotype and family number. The details on

each label were also entered in a logbook.

BREEDING CAGES

Cheap, expendable, cages for small numbers of mosquitos were provided by "Mono" paper cups. The tops were covered with netting and the mosquitos introduced through a star-shaped incision in the base, which either held an oviposition vial or was plugged with cotton wool (Plate III d). 190-ml capacity cups were used for single pairs and 300-ml capacity cups for up to about 10 pairs. Larger numbers of adults and colony material were bred in 18 cm cube "Barraud-type" "Terylene" net cages supported by corner tapes from a 20 cm metal frame (Plate IV g^{13}). Mosquitos were sorted into breeding groups while still in the vials and then put into an appropriate cup or cage, one group at a time. All the vial labels were transferred either to the side of the cup, or, in the case of the Barraud cage, on to a special label (Plate IV g^{12}).

Feeding of adults

For at least the first few days the mosquitos were provided with cotton pads soaked in dilute honey solution on the net tops of the cages. The paper cups were usually covered with wet cloths for several days prior to the offering of a blood meal when the females were 5 or 6 days old. Up to 18 cup cages were positioned under, or rested on, the writer's arm for 10-minute periods. The process was repeated several

times before cups containing females which had failed to engorge were discarded. The Barraud-cages were offered warm outdated human transfusion-blood through pig-gut membrane, using a slightly modified Ogden method (OGDEN, 1961). Four "Perspex" tubes passing through an electrically heated and stirred thermostatically-controlled water bath (Plate IV g^{9-11}) carried screw-threaded caps on the lower ends. Pig-gut membrane was stretched over these caps which were then filled with the blood and screwed in place. The weight of the water bath was carried by the wire-cage frame, the membranes pressed against the net of the cage. (A simpler modification could be designed to dispense with the heavy water bath by screwing the membrane caps directly on to a heat-conducting metal plate heated and thermostatically controlled in the manner of an electric iron). Before this machine was adopted or whenever it failed to achieve good engorgement, the writer's (or an assistant's) hand was introduced through the sleeve opening of the cage.

Oviposition and collection of eggs

Oviposition sites for the cup cages consisted of the standard shell vials lined with Whatman grade 1 filter paper and half-filled with water. These were pushed through the star-shaped incision in the base of the cups and the whole stood in wire racks of 8 (Plate III d). Oviposition sites for the Barraud cages were 100 ml narrow-type glass beakers

with the rim specially flared out to take a folded filter-paper cone. When the beakers were half-full of water, the filter-paper cones were completely wet with a very small water surface at the bottom. Eggs were allowed to remain damp for three days after oviposition, but free water was drained away. When almost dry, the filter-paper cones from the large cages were transferred to shell vials plugged with cotton wool. The cup oviposition-vials were simply plugged when the paper was nearly dry and the original adhesive labels of the parents transferred from the cups. The plugged vials of eggs were kept until used, or discarded after 4 months.

ROUTINE

A strict routine is essential to obtain the maximum advantage from the resources available, while avoiding when possible the necessity for Saturday or Sunday working. At the temperature stated above, eggs were normally hatched on Tuesday mornings. Pupation began on Saturday afternoon so that no more food was required after a final addition on Friday afternoon. First emergence of adults normally began during Monday afternoon, by which time all pupae would have been transferred to shell vials or cage beakers.

Examination of adults commenced on Wednesday and was completed with the final emergences by Friday, when pairs were selected and transferred to cup cages. Up to about 100 single pairs could be accommodated and these were normally

left covered with a damp cloth beneath a sheet of aluminium foil. The cloth was usually dry by Monday morning and removed. The females, which had had good opportunity of mating, were mostly ready to feed when offered a blood meal on Monday afternoon. Those failing to feed were again offered a blood meal on the following day. Experience showed that females refusing blood twice were usually unfertilized - in most cases because the male had died - so that they were discarded. After females had successfully bloodfed, their cup-cages were set on water vials in the racks and provided with honey pads.

Eggs were normally laid by Friday and so were ready for hatching on the following Tuesday, giving a minimum practical generation time of 3 weeks. Where possible at least 10 pairs of any mating type were set up, but only the best egg batch (i.e. from the pair with the greatest selective value), in the case of a continuous variable, or that with the most eggs among pairs of equal value, was normally used. Only rarely did every female of a group of pairs feed and of those that did feed some often laid no eggs. Where the choice was limited, several egg batches were obtained from the best available pairs to ensure a reserve supply. Such repeated feedings reduced the possible number of new single pairs. Colonies were run on a rota of about five weeks in every 3 months during which about 3 egg batches were collected. Thus three times as many could be run intermittently as against

continuously. Colonies were normally given an opportunity to feed on Tuesdays and Fridays.

The routine may be summarized as follows:

Monday - Pupae transferred to vials, single pairs offered blood meal, colony eggs collected.

Tuesday - Eggs hatched, pupae transferred to vials, remainder of single pairs offered blood meal, colonies offered blood meal.

Wednesday - Final pupae transferred to vials, examination of adults, larvae fed.

Thursday - Examination of adults, larvae fed.

Friday - Examination of adults concluded, selected pairs caged, single-pair eggs collected, larvae fed, colonies offered blood meal.

GENETICAL METHODS

GENERAL

Larvae and pupae were not routinely examined, neither was any attempt made to count total eggs or percentage hatch, since the time and labour involved would have considerably reduced the amount of material handled. All adults from the initial batch and subsequent single-pair families were carefully checked for variation in abdominal scale pattern, width of white bands on the metatarsi, colour and patterning of the dorsal aspect of the thorax, eye colour and palps.

Details of wing-venation and scaling were not studied.

Any obvious or suspected mutants observed for the first time were bred together if numerous or outcrossed if few, and standard procedures of genetic analysis followed. Analysis was often frustrated by the failure to obtain offspring and the difficulty of repeating the cross with the original material. The limiting factor was the number of families or strains that could be handled in a single week; it was difficult always to have the desired material available at the same time.

CLASSIFICATION OF ABDOMINAL COLOUR

Much variation permitted simple description and scoring as present or absent. Abdominal colour was an exception, almost every subtle intergrade from completely white to completely black being noted. A classification covering all possible variations and a numerical measure of paleness was required. The writer had earlier devised such a scheme (McCLELLAND, 1960b) giving 11 pattern grades ranging from F (representing subspecies formosus as defined by MATTINGLY, 1957) through G, H, J, K, L, M, N, P to Q (representing extreme var. queenslandensis) with a somewhat arbitrary paler grade R. These grades were based on the number of segments that were medially pale, defined as having a continuous pattern of pale scales extending from the basal to apical margin. Thus F = 0, J = 1, K = 2, etc. to Q = 7. The

intermediate Grade H was necessary as corresponding to the type form defined by MATTINGLY (1957) referred to henceforth as the wild-type. Grade G was a necessary intermediate between F and H.

COLOUR GRADE AND VALUE

In the present work it soon became apparent that the 11 grades, which had originally sufficed, were inadequate to describe many of the patterns observed in the wider range of material available. The reason for this was that the original grading system ignored tergites which were brindled - or had a non-contiguous pattern of pale scales (in addition to basal bands and lateral spots), so that grade J, where the first tergite was medially pale and only the second brindled, was also the grade for examples, some of which really appeared paler than K or L having all the tergites beyond the first brindled. The grading scheme was therefore revised to take brindled tergites into account. The number of medially pale tergites was denoted by letters corresponding to the earlier scheme and the number of other tergites that were brindled denoted by a subscript as shown in Table II. Plate I illustrates diagramatically some representative colour grades. Logically G should be F^1 and H should be J^0 (or J^1 etc. should be H^1 etc., but their retention is justified by further sub-division (vide infra). The other theoretical grades in the top line are left blank because they have never been observed.

TABLE II

NUMBER OF TERGITES MEDIALLY PALE SCORES 2 x	NUMBER OF TERGITES BRINDLED SCORES 1 x							
	0	1	2	3	4	5	6	7
0	F 0	G 1						
1	H 2	J1 3	J2 4	J3 5	J4 6	J5 7	J6 8	
2	K0 4	K1 5	K2 6	K3 7	K4 8	K5 9		
3	L0 6	L1 7	L2 8	L3 9	L4 10			
4	M0 8	M1 9	M2 10	M3 11				
5	N0 10	N1 11	N2 12					
6	P0 12	P1 13						
7	Q 14							

TABLE II. Explanatory scheme for the colour grades and values. Notes - The figures at the bottom of each square are "colour values". These are obtained by adding twice the number of medially pale tergites to the number of remaining tergites that are brindled. — G is further subdivided into G and G- . H is further subdivided into H-, H, H-ap and H ap. — Q is further subdivided into Q- , Q and Q+. See text for definition of medially pale or brindled tergites.

If two brindled tergites are deemed equivalent to one medially pale it is possible to compute a numerical measure of paleness. Thus, twice the number of medially pale tergites is added to the number that are brindled, giving the small figures in Table II henceforth named colour values. With the proviso of unimodality it is therefore possible to determine the mean colour value of a population. In the preparation of histograms the colour values can be conveniently treated as 8 groups of two, while the original data remains in 30 grades as a check on the validity of the grouping. In the case of selection for colour where sometimes families are very uniform, further sub-division is necessary. Hence G- is almost as dark as F but excluded from grade F by having at least a single pale scale on the first tergite. H- represents a medially pale but black-speckled first tergite and ap denotes a clearly defined apical band of pale scales. Q-, Q and Q+ differ in the breadth of pale scaling. Q+ is probably close to what was earlier termed R, and so probably deserves a higher colour value. For this reason the colour value grouping in the histograms extends to 15.

METHOD OF SCORING FOR COLOUR

Mimeographed proformas listing all the 37 colour grades and sub-grades, together with space for family data, remarks on other variation, etc. were used to score the colour grade of every individual offspring of each family. Such completed proformas constitute the basic data on colour pattern

throughout the present work. Where the initial material of any strain showed some variance in abdominal colour grade, the individuals were ranked in increasing paleness according to the following sequence :

F< G - < G < H - < H < H-ap < H ap < J1 < K0 < J2 < K1 < J3 < L0 < K2 < J4 < L1 < K3 < J5 < M0 < L2 < K4 < J6 < M1 < L3 < K5 < N0 < M2 < L4 < N1 < M3 < P0 < N2 < P1 < Q- < Q < Q+(R).

The most extreme and successively less-extreme pairs from such a rank constituted selection for pale or dark abdominal colour. The process was repeated with the progeny of the most extreme pair laying viable eggs but abandoned if there were no clear response in the F_2.

TECHNIQUE FOR FORCE-MATING

A method of inducing copulation in Aedes mosquitos was first described by McDANIEL and HORSFALL (1957). BAKER et al. (1962) have described a modified technique for Anopheles which embodies some improvements independently devised by the writer. In essence, force-mating involves the juxtaposition of the genitalia of intact anaesthetized females with those of decapitated, unaesthetized, males.

In the present work two additional types of "sucking-tubes", connected to the vacuum system described above, facilitated handling of the males and females. One such tube with a terminal orifice of 0.22 mm. was used to catch and hold

male mosquitos while the legs and head were removed with micro-scissors (Plate IV h). This avoided the necessary recovery period following anaesthesia with CO_2 (as practised by Baker et al.). Males so prepared were fixed, venter up, with a water-soluble adhesive ("Seccotine") to numbered sectors of a perspex disc mounted on an iron ring (Plate IV i). This gives better stability than the microscope slides used by the other workers. The females were first etherized in the manner described earlier and then picked up and held by applying a third type of sucking tube (terminal orifice 0.5 mm diameter) to the mesonotum, as described by Baker et al. The female is held, venter up, at an angle of about 130° to the male and the genitalia apposed (Plate IV i).

Should copulation not occur immediately the female is tried with a rapid succession of males. This is simply done by rotating the disc with one hand, holding the female pipette with the other while focussing the microscope by foot. Females are given a blood meal en masse before force-mating to avoid waste of time in mating a female which subsequently refuses to feed.

Engorged, force-mated, females are returned to individual pupal vials. Two days later they are transferred to clean pupal vials with a strip of filter-paper up the side and extra water just covering the cotton pad. Eggs collected in these tubes are treated in the same manner as those in cup-cage oviposition vials.

In the present study force-mating has been used for routine maintenance of species not normally mating in the laboratory, for mating a single rare mutant female to several males or several females to a single mutant male, where there was reason to doubt the viability of the mutant, and for inter-specific matings. In all these cases successful transference of seminal fluid can usually be confirmed by observing either or both (a) a slight distension of the terminal segments of the female, and/or (b) a silk-like thread of seminal fluid connecting the two genitalia when the male and female are pulled apart.

PHOTOGRAPHY

The abdomens of pinned mosquitos shrink to such an extent that comparison with living material is extremely difficult especially with regard to subtler colour differences. Photography was therefore adopted as a routine alternative to pinning A. aegypti and related species; these, being largely black and white, have the added advantage of being adequately portrayed in monochrome.

For photography a monocular body (Plate IV f) was substituted for the stereomicroscope (Plate IV e). Either a 48 mm. 25 mm. or 18 mm. "Beck microstigmar" photographic objective with iris diaphragm was used in conjunction with a x6 "Beck" projection eyepiece. An adaptor (Plate IV f^6) carried a Zeiss "Contaflex III" 35 mm. camera.

The photographic stage consisted of a cylindrical perspex box connected to the vacuum system described earlier. This box was held in a universal mounting at the end of an extensible arm (Plate IV f^5) which could be swung in position under the microscope. The top of the box, a loose 6.5 mm. thick disc of transparent perspex, was held secure by the partial vacuum.

The subject mosquito was etherized and held by suction against a 0.5 mm. diameter hole bored in the centre of the disc (Plate IV f^7). The floor of the box could be covered with suitably coloured paper as a background. The important feature was that the shadows of the mosquito cast by the two lamps (Plate IV f^8) were invisible on the supporting perspex disc and, on the background, fell to either side of the field of view. As the two lamps were not identical, the rheostats of each had to be separately adjusted to give equal intensities at about 30-48 watts output. Heat-absorbing and pale blue filters provided a cool light of approximately daylight hue.

All photographs of abdomens or whole mosquitos (Plates VI - XI, XVII and XVIII i,k.) were taken using the 48 mm. objective stopped down to f 11. and no draw tube extension. All those of thoraces, tarsi and other parts (Plates V, XII - XVI & XVIII g,h,j.) were similarly taken with the 48 mm. objective, but with full draw tube extension, with the exception of Plate XVI d which was taken through the 18 mm.

objective with no draw tube extension. "Kodak panatomic-X" film, developed with "M. & B. Promicrol", was used for all monochrome with typical exposures of 7-20 seconds. Plate V (upper) was taken with "Kodachrome II" film with exposure of 150-240 seconds. Plate V (lower) was taken on the same set-up using dark-ground type illumination. The photographs of other slide-mounted material in Plate XIX were taken with the same camera, eyepiece and objectives as before, but used on a standard "Baker" high-power microscope stand with condenser illumination.

Wherever possible, photographs of the dorsal abdomen were taken of freshly emerged mosquitos, as soon as the initial distension due to swallowed air had subsided. This takes advantage of the fact that the tergites of newly emerged mosquitos are almost flat and expanded. The lateral spots are therefore visible in dorsal view, whereas, after about six hours, the sides of the tergites curve round ventrally. In old or starved individuals the lateral spots are only visible ventrally. This accounts for the possibly unfamiliar appearance of the photographs.

PART III

MUTANTS, LINKAGE AND VARIABILITY IN *AEDES AEGYPTI*

MUTANTS, LINKAGE AND VARIABILITY IN AEDES AEGYPTI.

INTRODUCTORY

From the foregoing account it is apparent that almost the only published formal genetic analyses in A. aegypti, involving more than simple breeding experiments, is the work of Craig and his associates at Notre Dame, U.S.A. summarized in Table III together with a few unpublished modifications[1]. The estimate of 25% crossing-over between the factor for dieldrin resistance and yellow by KAHN and BROWN (1961) and Macdonald's demonstration of sex-linkage for the factor responsible for susceptibility to Brugia malayi (MACDONALD, personal communication) are the only other linkage data that could be added.

In view of the variability of colour throughout the mosquitos, in contrast to the relative uniformity of anatomy (see SNODGRASS, 1959), it is not surprising that most of the colour mutants found by CRAIG and VANDEHEY (1962) are highly viable, penetrant and useful "marker genes", whereas a high proportion of the structural mutants they found were subvital,

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[1] G. B. Craig. Semi-Annual Report to U.S. Army Biological Laboratories September 1961 - March 1962

TABLE III

SITE	SYMBOL AND MUTANT NAME	NO. OF LOCI	DOMINANCE WITH RESPECT TO WILD-TYPE	LINKAGE GRP.	PERCENT CROSSOVER	REFERENCES
head	hk hook-proboscis	?	?	?	-	4
	lab labella-less	?	?	?	-	4
thorax	G Gold	1	D(♀), r(♂)	II	6.5 from y	3,5
abdomen	W White	1	semi-D	II	5.9 from y	3,5
	s spot	1	r	II	6.5 from y	3,5
	Hf Half-genitalia	1	D	I	-	4
palp	B Bulb	1	D (♂ lmtd)	I ?	-	4
	blp black-palp	1	r	III	-	3,5
	wa wart	1	r	II	1.8 from s	4,5
	kn knobbed	1	r	(A)	-	4
	ki kink	?	?	?	-	4
	5-j 5-jointed	?	?	?	-	4
	sp speck	1	r (♀ lmtd)	?	-	3
antenna	bu bulbous	1	r (♂ lmtd)	(A)	-	4
	k knob	1	r (♂ lmtd)	(A)	-	4
	fu fused	1	r (♀ lmtd)	(A)	-	4
	dr droop	1	r	(A)	-	4
	co compressed	1	r	III	5.8 from blp	5
wing	N Notch	1 ?	semi-D ?	?	-	4
	bt bent	?	?	?	-	4
	h halteres	?	?	?	-	3
	lb lobe	?	?	?	-	4
	nt notch-trail	?	?	?	-	4
	scr1-4 scale-row 1-4	?	?	?	-	4
	cv crossveinless ci cubitus-interruptus	1 ?	?	?	-	4
	av anal-vein	?	?	?	-	4
	ar-1 abbreviated-radial-1	1 ?	r ?	?	-	4
	exv extra-crossvein	?	?	?	-	4
leg	blt black-tarsi	1	r	III	-	3
	li lightfoot	1	r (♂ lmtd)	?	-	3
	cl club-foot	?	?	?	-	4
	sw swollen	?	?	?	-	4
	wi withered	1	r	(A)	-	4
	br broken	1	r ?	(A)	-	4
larva	y (also y^m, y^b ?) yellow	1	r	II	-	1
other	min miniature	1	r	II ?	-	4,5
	MP Males-predominate	1	D ?	I ?	-	2,5
TOTAL DATA IN EACH CLASS 38		24	23	12 (19)	5	

TABLE III. The results of (1) CRAIG & GILLHAM (1959), (2) CRAIG et al. (1960), (3) CRAIG & VANDEHEY (1962), (4) VANDEHEY & CRAIG (1962), (5) CRAIG (unpublished), summarized.

impenetrant or inconstant in expression (VANDEHEY and CRAIG, 1962). In the latter, heritability was demonstrated from repeated isolation in the same line, but the mechanisms involved defied resolution. Thus, out of 38 so-called mutants, only 21 are definitely established as monofactorial. Nine out of the 19 in which the degree of dominance is known, are definitely assigned to one or other of the three linkage groups, although the remainder are tentatively designated sex-linked or autosomal. They found no instance of partial sex-linkage but were able to obtain 5 measurements of linkage distance, three to the y locus and one to the s in Group II and one between co and blp in Group III. Miniature - min is queried in Table III, as although VANDEHEY and CRAIG (1962) showed that it assorted independently from white abdomen (they give a χ^2 of 14.38 for deviation from 9:3:3:1 ratio; but min homozygotes clearly fall short of 25% expectation and a straight test for linkage using their figures gives χ^2 = 1.97), in a later unpublished report[1] it is nevertheless placed in Group II.

Several of Craig's mutants were used in the present work. These are as follows:

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[1] G. B. Craig. Semi-Annual Report to U.S. Army Biological Laboratories September 1961 - March 1962

W - White. This is stated (CRAIG and VANDEHEY, 1962) to be a single, semi-dominant, gene increasing the amount of pale scaling on the abdomen, linked to y in Group II, with about 6% crossing-over. The Tübingen strain, used in Craig's work, homozygous for W, is the AO strain of the present work. Craig and VandeHey point out that their data conflict with the writer's earlier results (McCLELLAND, 1960b) claiming that the pattern of white scaling on the abdominal tergites is under multifactorial control.

s - spot. CRAIG and VANDEHEY (1962) describe this as a single, fully penetrant, recessive factor linked to y in Group II with about 6% crossing-over. The male is characterized by absence of the lateral spots on segments 1 to 7. In the female the lateral spots are enlarged and oblique, although sometimes male-type. In addition, there is a general increase in the pale scaling of the abdomen. It is pointed out that linkage tests between s and W are difficult because both factors increase abdominal pale scaling so that segregants are difficult to separate. The BLTS strain received from Craig was homozygous for this factor. Craig records that the writer had shown s to be allelic with the gene causing "white-spot" (McCLELLAND, 1960b).

G - Gold. According to CRAIG and VANDEHEY (1962) this factor, causing the normally black scaling of the mesonotum to be pale gold or yellow-coloured, is inherited as a single factor showing dominance in females but recessive in males.

G is also linked to y in Group II with about 6% crossing over. Craig and VandeHey make the point that G W and s all show about the same crossover frequency with y, so that at least two must be very closely linked, and possibly all three. Strain AO was also homozygous for G.

blt - black tarsi. This is described by CRAIG and VANDEHEY (1962) as a single recessive factor, causing reduction in the white banding of the tarsi apparently identical to var. atritarsus of EDWARDS (1941). As it is neither linked with sex or y, it is assigned to linkage group III. Both strains BLTS and BLP of the present study were homozygous for this factor.

CRAIG and VANDEHEY (1962) reported in addition 4 other "colour" mutants affecting the amount of pale scaling on the palps (2), legs and halteres, but no clear data on inheritance was given.

In the present study linkage information has been obtained for a further 7 loci including sex and several distinct alleles isolated at the s and blt loci. Evidence is also given suggesting that W is in fact an allele at the s locus. Heritability of another 5 characters is demonstrated and comment is made on 18 other variations, some of which are probably similar to those described by Craig and VandeHey.

DESCRIPTION OF MUTANTS AND INHERITANCE

FACTORS OF KNOWN LINKAGE GROUP

LINKAGE GROUP I

1. re - red eye. A partially sex-linked, recessive, fully penetrant mutant affecting eye colour, isolated spontaneously from strain GA.

Description

The black pigment of the wild-type eye (Plate V a,h) is not formed, or is formed very slowly, so that the freshly emerged adult eye is a deep red colour (Plate V d). An associated effect is that all or part of the eye surface appears rather shiny as if wet, especially round the edge of each facet. Although the adult eye gradually darkens with age the shininess persists so that old dark-eyed adults may still be scored with confidence. The initial colour of the freshly emerged adult and the rate of subsequent darkening shows some inter- and intra-family variation, but the variation in expression is small compared with the departure from the wild-type colour.

The pigment change is visible from the second stage larva onwards, but is most easily differentiated by naked-eye at the young pupal stage, before darkening of the pupal integument, when it appears bright scarlet.

The histology of the wild-type and mutant eye has not been compared, but gross examination of xylol-cleared material (Plate V k) suggests that the shiny appearance is due to a loss of pigment in the outermost layer of the iris pigment cells. The lack of any obvious behaviour anomaly in the mutant adults suggests that vision is functionally normal.

Inheritance

All crosses between red-eyed and wild-type adults give wild-type offspring. F_1 matings and backcrosses are summarized in Table IV (A—E). The total F_2 segregations (A,B), 1428 wild-type: 407 re departs significantly from a 3:1 ratio ($\chi^2 = 7.78$ $P<.01$ $n=1$) although the figure for the males separately, 734 wild-type : 210 re is not significantly different ($\chi^2 = 3.82$ $P<.10>0.05$) and the female data 694 wild-type : 197 re are only just significantly different ($\chi^2 = 3.90$, $P<0.05 > 0.02$). The total backcross segregations (Table IV C,D,E) do not depart significantly (at the 5% level) from a 1 : 1 ratio for both the males, 554 wild-type : 535 re ($\chi^2 = 0.33$) and the females, 493 wild-type : 447 re ($\chi^2 = 2.25$). The slight excess of males is not unusual in the species.

When the segregation data from the two reciprocal parental-type matings are separately compared, however, there is clear evidence of partial sex-linkage. F_1 matings from a maternal red-eyed cross give nearly equal numbers of re and

TABLE IV

	PARENTS		number of families	PROGENY			
	MOTHER	FATHER		+ ♂♂	re ♂♂	+ ♀♀	re ♀♀
A.	+ m / re m normal (re parent)	+ M / re m normal (re mother)	c. 15	394	12	196	163
B.	+ m / re m normal (re parent)	re M / + m normal (re father)	c. 20	340	198	498	34
C.	re m / re m red-eyed	+ M / re m normal (re mother)	13	425	33	24	343
D.	re m / re m red-eyed	re M / + m normal (re father)	c. 10	26	386	365	26
E.	+ m / re m normal (re parent)	re M / re m red-eyed	c. 8	103	116	104	78

TABLE IV. Crosses with re - red eye, showing sex-linkage.

wild-type daughters but very few re examples among the sons. F_1 matings from a paternal red-eyed cross give nearly equal numbers of re and wild-type sons but very few re daughters. Backcrossing an F_1 male with a red-eyed mother to a red-eyed female produces mostly re female and wild-type male offspring. Backcrosses of an F_1 male with a red-eyed father to a red-eyed female give mostly re male and wild-type female offspring. Backcrosses of any F_1 female to a red-eyed male give approximately equal numbers of both re and wild-type sons and daughters.

This occurrence of partial linkage with sex exactly parallels the case of white-eye in C. p. molestus (GILCHRIST and HALDANE, 1947). On the assumption of a similar mechanism of sex determination in A. aegypti the gene re may occur on the same chromosome as either the dominant "factor" for maleness, M, or the recessive "factor" for femaleness, m. A red-eyed male is therefore $\frac{re\ M}{re\ m}$ and a female $\frac{re\ m}{re\ m}$. More important, a male heterozygous for re can be of two alternative genotypes, $\frac{re\ M}{+\ m}$ or $\frac{+\ M}{re\ m}$. If there is crossing over between the re locus and the M "locus", either type of heterozygous male will produce 4 different gametes with respect to these loci.

A heterozygous female, being homozygous m, will produce only two sorts of gametes + m or re m, just as the red-eyed male, homozygous re, will produce only re M and re m gametes. This explains the three possible results of

backcrossing heterozygous re to its homozygote (Table IV, C,D, E.). Linkage information is therefore only obtainable from backcrosses using heterozygous males, and less precisely from F_2 data. Recombination between re and M using the data from backcrosses (C) and (D) (Table IV) is proportional to $\frac{33 + 24 + 26 + 26}{1628} = 6.70\%$.

2. ru - rust eye. A partially sex-linked, recessive, fully penetrant mutant affecting eye colour, isolated spontaneously from strain VL.

Description

As in re the wild-type black eye pigment is not formed, or is formed very slowly. The eye colour of the freshly emerged adult varies from a dark orange brown to a bright rust (Plate V c). As in re the colour darkens with the age of the adult and, because there is no associated change in the appearance of the eye, older adults are not always scored with confidence. In the young pupa the eye appears dark brown and is not therefore as easily separated from wild-type as re . Material in alcohol or xylol (Plate V j) is almost indistinguishable from re.

Inheritance

All crosses between rust-eyed and normal adults give normal offspring, as also do crosses between rust-eyed and red-eyed. ru is therefore recessive and not an allele of re.

The results of backcrossing heterozygous males, shown in the case of re to give maximum linkage information, are given in Table V (F,G.). The other crosses were omitted. Total segregation of 504 + : 561 ru does not differ significantly from a single-factor 1 :1 expectation ($\chi^2 = 3.06$, $P > 0.05$). The difference is even less for males and females separately, but obviously different within each separate cross. Rust-eyed males are clearly in excess in the progeny of (F) in which the paternal grandfather was ru and deficient in the progeny of (G) in which the paternal grandmother was ru. Rust-eyed females show the opposite trend, clear evidence again of partial sex-linkage. Recombination between ru and M is proportional to $\frac{51 + 140}{660} = 28.9\%$ in the (F) backcrosses, and $\frac{31 + 39}{405} = 17.3\%$ in the (G) backcrosses, an obviously significant difference ($\chi^2 = 18.3$).

The double recessive, ru re.

Red-eyed females were crossed with ru males and the F_2 searched for the recombinants, ru females and re males. Assuming that some of these might also be heterozygous for the other gene, single pairs were set up. Both sexes of

TABLE V

	PARENTS		number of families	PROGENY							
	mother	father		males				females			
				+ +	+ re	ru +	rure	+ +	+ re	ru +	rure
F.	$\frac{ru + m}{ru + m}$	$\frac{ru + M}{+ + m}$	15	140	-	332	-	137	-	51	-
G.	$\frac{ru + m}{ru + m}$	$\frac{+ + M}{ru + m}$	8	188	-	31	-	39	-	147	-
H.	$\frac{rurem}{rurem}$	$\frac{ru + M}{+ rem}$	11	40	13	128	0	0	124	5	22
I.	$\frac{rurem}{rurem}$	$\frac{+ reM}{ru + m}$	11	2	106	14	34	40	13	130	0
J.	$\frac{+ rem}{ru + m}$	$\frac{ureM}{rurem}$	10	34	259	286	41	36	229	197	33

TABLE V. Crosses with re - red eye and ru - rust eye.

the double recessive appeared in one out of three such progenies and a true breeding line was started.

The eye of the doubly recessive adult is much paler than either re or ru, approaching a bright pink which is readily visible without magnification (Plate V e). There is no associated shininess as in re, but, as there is practically no darkening with age, scoring is always reliable. Behaviour and vigour seemed normal, although it is possible that the double recessive, and ru to a lesser extent, are more susceptible to desiccation. The eye colour of the young pupa is very pale pink, and that of adult material in alcohol or xylol pale red and more transparent than re or ru (Plate V l).

Three-point estimation of linkage between ru, re and M.

To the double recessive ru re females were backcrossed doubly heterozygous males from the cross re x ru (Table V H), or the reciprocal, ru x re (Table V I). Possible gene sequences are ru re M, re ru M and re M ru. The greater crossover value between ru and M compared with re and M rules out the second arrangement. In mating H, where ru and M are linked, the rarest classes expected, the double crossovers, will be ru re males and + + females assuming the first arrangement, and + re males and ru + females assuming the third arrangement. The rarest observed classes are ru re males and + + females. Similarly in mating I the rarest

classes expected will be + + males and ru re females assuming the first arrangement, and ru + males and + re females assuming the third. The rarest observed classes are + + males and ru re females. There is thus no doubt that the sequence is ru re M.

Crossovers between ru and re will be revealed by + + and ru re males and females in both crosses. The crossover value is therefore

$$\text{c.o.v.}_{\text{ru} \longrightarrow \text{re}} = \frac{100\ (40 + 22 + 2 + 34 + 40)}{671}\% = 20.57\%.$$

Crossovers between re and M will be revealed by + re and ru re males, ru + and + + females in cross H; ru + and + + males, + re and ru re females in cross I. Hence the crossover value is

$$\text{c.o.v.}_{\text{re} \longrightarrow \text{M}} = \frac{100\ (13 + 5 + 14 + 2 + 13)}{671}\% = 7.00\%.$$

Crossovers between ru and M are revealed by + + and + re males of cross H and females of cross I, ru + and ru re females of cross H and males of cross I. To the percentage of these must be added twice that of the total of double crossovers. Thus

$$\text{c.o.v.}_{\text{ru} \longrightarrow \text{M}} = \frac{100\ (40 + 13 + 40 + 13 + 5 + 22 + 14 + 34)}{671}\% + \frac{200 \times 2}{671}\% = 27.57\%.$$

Further estimate of linkage between ru and re.

Backcross of doubly heterozygous females $\frac{ru + m}{+ re\ m}$ to doubly recessive males (Table V J) gives the same expectations as for an autosomal backcross. Crossover classes are + + and ru re males and females. Therefore

$$c.o.v._{ru \longrightarrow re} = \frac{100\ (34 + 41 + 36 + 33)}{1115}\ \% = 12.83\%$$

Homogeneity of data on linkage between re and M.

To the backcrosses of heterozygous re males to re females totalled in Table IV can be added those in Table V. Together these comprise 44 single pair families, detailed in Table VI (98 offspring from a multiple mating (in D) which included six gynandromorphs (vide infra) were omitted) which afford a check on homogeneity. Recombination between re and M can be calculated from male and female progeny separately, both in coupling (D and I) and in repulsion (C and H), as follows: coupling males 6.49%, coupling females 4.81%, repulsion males 7.20% and repulsion females 5.60%. The χ^2 for the 4 groups is 3.315 ($P > 0.3$, $n = 3$), χ^2 for all the males compared with all the females is 2.70 ($P > 0.1$, $n = 1$) and χ^2 for all coupling families compared with all repulsion families is 0.663 ($P > 0.3$, $n = 1$). The amount of crossing-over in each family similarly shows no correlation with either sex ratio or family size, and the data for re/M recombination are clearly homogeneous.

TABLE VI

TYPE OF CROSS		NON-CROSSOVERS			CROSSOVERS			TOTAL		
		♂♂	♀♀	tot.	♂♂	♀♀	tot.	♂♂	♀♀	tot.
coupling cross	D 1	21	23	44	0	0	0	21	23	44
	2	18	16	34	2	1	3	20	17	37
	3	26	12	38	1	0	1	27	12	39
	4	23	8	31	1	0	1	24	8	32
	5	9	10	19	1	0	1	10	10	20
	6	4	4	8	0	0	0	4	4	8
	7	26	15	41	0	0	0	26	15	41
	8	4	4	8	0	2	2	4	6	10
	9	52	58	110	9	3	12	61	61	122
	10	23	23	46	1	1	2	24	24	48
	11	46	41	87	9	4	13	55	45	100
	12	69	60	129	3	12	15	72	72	144
	13	64	41	105	4	0	4	68	41	109
	14	40	28	68	2	1	3	42	29	71
	I 1	14	1	15	1	0	1	15	1	16
	2	24	26	50	2	1	3	26	27	53
	3	13	14	27	1	1	2	14	15	29
	4	8	9	17	0	0	0	8	9	17
	5	13	14	27	1	0	1	14	14	28
	6	14	6	20	1	0	1	15	6	21
	7	15	13	28	0	0	0	15	13	28
	8	14	13	27	1	2	3	15	15	30
	9	15	10	25	1	1	2	16	17	27
	10	10	17	27	1	0	1	11	17	28
	11	28	23	51	4	0	4	32	23	55
repulsion cross	C 1	38	16	54	1	0	1	39	16	55
	2	30	29	59	1	0	1	31	29	60
	3	23	21	44	3	3	6	26	24	50
	4	52	54	106	4	0	4	56	54	110
	5	53	37	90	3	2	5	56	39	95
	6	26	40	66	2	4	6	28	44	72
	7	75	79	154	4	3	7	79	82	161
	8	53	49	102	0	0	0	53	49	102
	H 1	23	21	44	1	3	4	24	24	48
	2	5	10	15	3	3	6	8	13	21
	3	14	19	33	1	1	2	15	20	35
	4	18	16	34	0	3	3	18	19	37
	5	4	11	15	3	2	5	7	13	20
	6	4	12	16	0	0	0	4	12	16
	7	8	8	16	1	0	1	9	8	17
	8	22	25	47	2	0	2	24	25	49
	9	16	24	40	1	1	2	17	25	42
	10	9	14	23	1	0	1	10	14	24
	11	17	10	27	3	0	3	20	10	30
TOTALS	44			2067			134			2201

TABLE VI. Single family data for all crosses with re - red eye.

Evidence of possible chiasma interference

From the three-point crosses H and I in Table V the probability of crossover between re and M is 0.0701, and between re and ru is 0.205. The probability of a double crossover between ru and M if the events are independent is thus 0.0144. Out of a total of 671 there were observed 2 double crossovers (both + +) as against an expected of 671 x 0.0144 = 9.66. In this case the standard error approximates the square root of the expected value = 3.11, and is less than half the difference between the values observed and expected. Observed chiasma frequency is thus significantly lower than expected.

3. pa - pale abdomen. A partially sex-linked, incompletely recessive, highly penetrant gene of variable expression affecting the amount of pale scaling on the abdomen. Spontaneous isolate from strain PR, with probable alleles in other strains.

Description

In its fullest expression the abdominal dorsum is extensively pale scaled reaching colour grade Q+ in the females, a somewhat less extreme example is illustrated in Plate VI a. The pale scales are not as white nor as densely

packed as those of the basal bands and lateral spots which remain clearly visible. The ventral abdominal surface is black banded, though not as extensively as in the wild-type (Plate VI e,f.). An associated effect in the PR strain is a great broadening of the median paired lines of the scutum (Plate XIII f) and considerable paling of the dorsal surface of the femora vide infra. The amount of pale scaling tends to be less in the males (Plate VI g), where, as in the less pale females, there is a tendency in some individuals for the pale scaling to be broader and more dense near the base of the tergite (Plate VI b); in others the pale scaling is simply more diffuse (Plate VII i).

Inheritance

The full expression of Q+ was not seen among a sample of 81 females hatched from the colony eggs as received, but there was a single Q female. No male was paler than H. The results of selection for palest expression through 4 generations of single-pair, brother-sister mating are given in Fig. 1. One of the males from the last family, colour grade J6, was then outcrossed to a dark female, colour grade G, of strain GA, giving an F_1 of grade H ap to J4 (Plate VII j) and the single-pair selection repeated for 5 generations (Fig. 2). The results of the two selections may only be reconciled with monofactorial control, if the major gene involved is subject to considerable genetic or

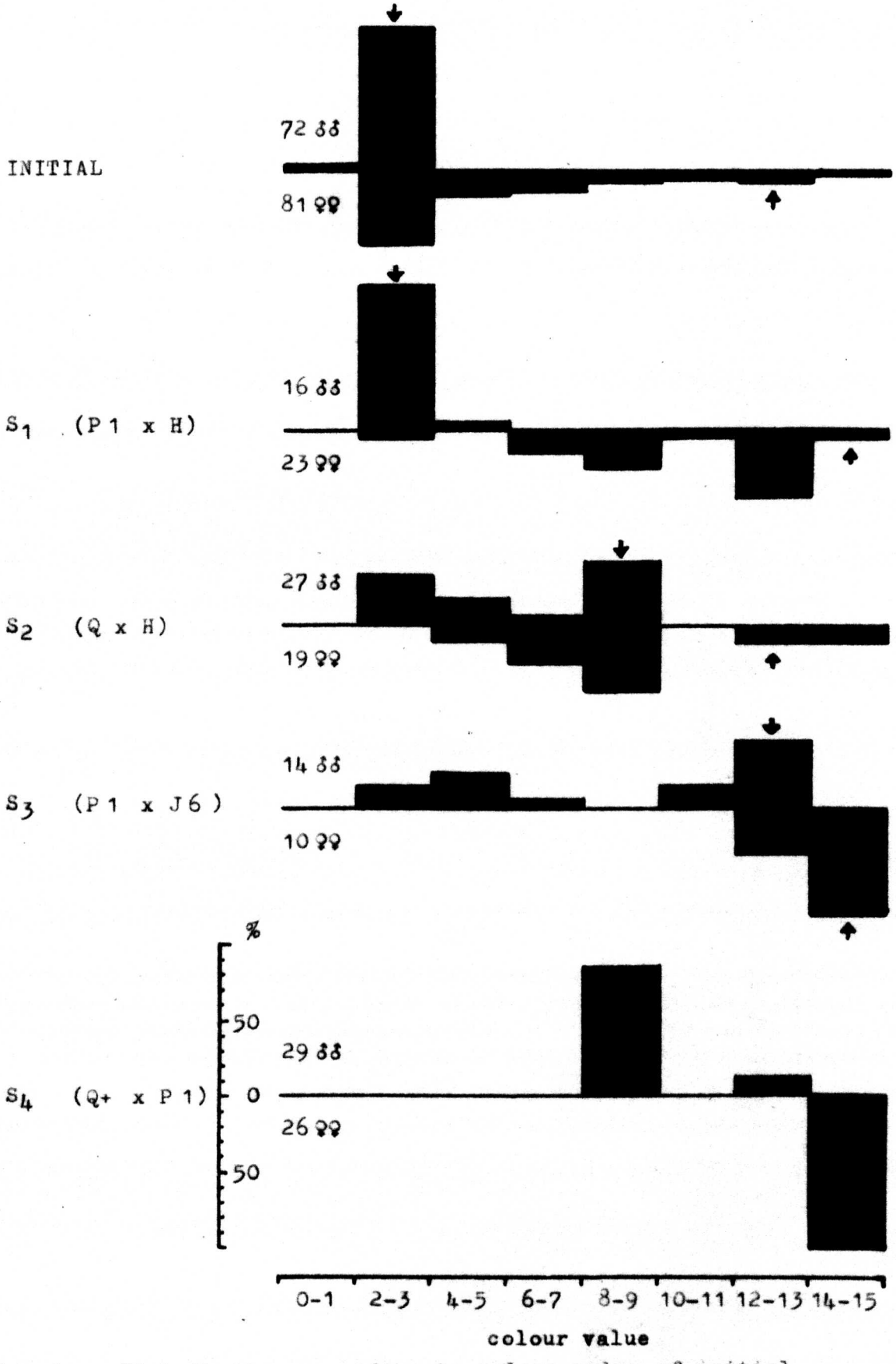

Fig. 1. Distribution according to colour value of initial population of PR and the result of selection for paleness.
Note - arrows indicate colour value of parents of next generation.

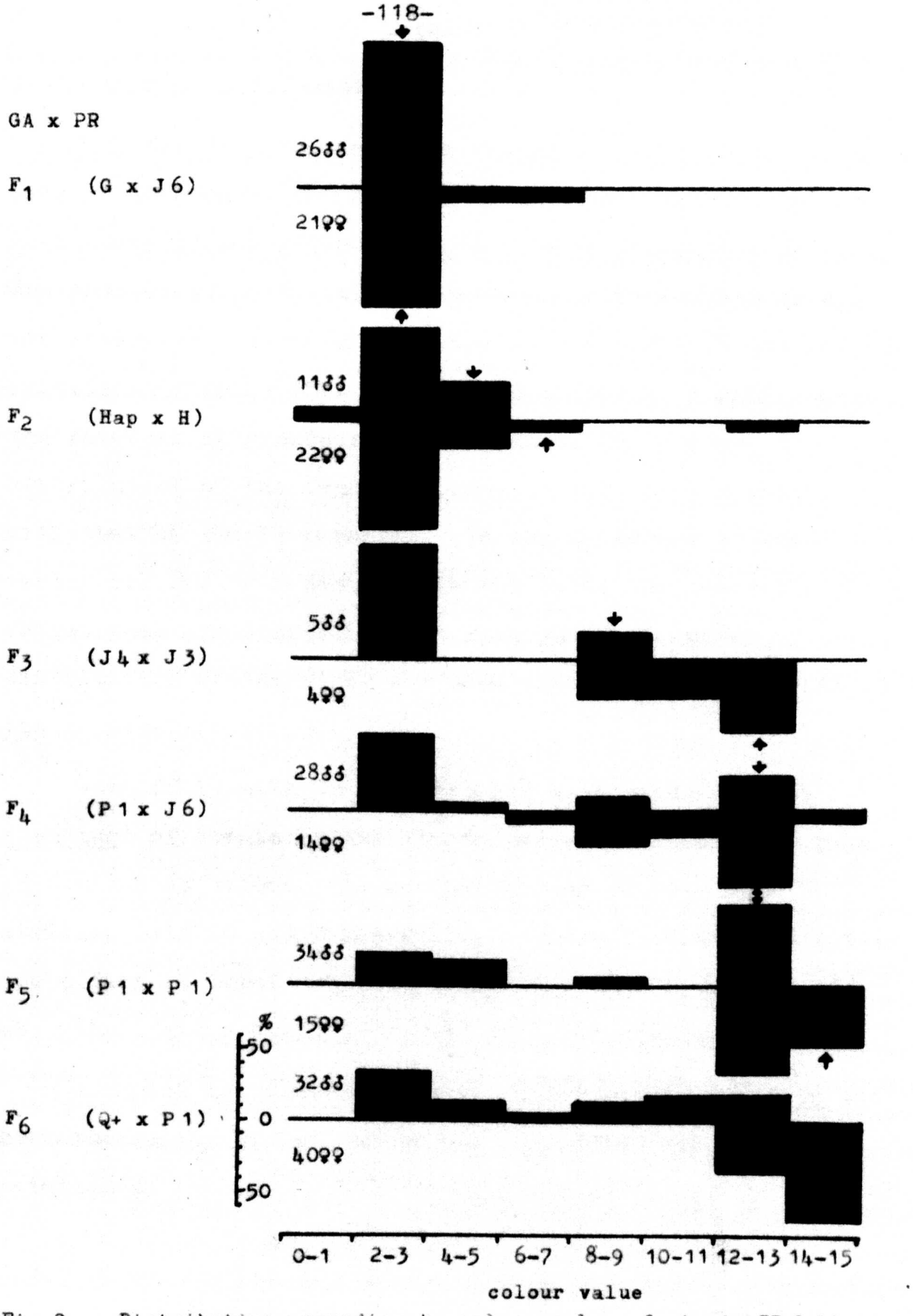

Fig. 2. Distribution according to colour value of strain PR following outcross to grade G of strain GA and subsequent selection for paleness. Note - see Fig. 1 note.

environmental modification.

The two F_1 offspring from reciprocal outcrosses of fully pale PR to grade H (wild-type) EN were essentially similar. As a test for sex linkage, males of each reciprocal F_1 were backcrossed to fully pale PR females. The results of all the crosses are represented graphically in Fig. 3 and set out in detail in Table VII. In the backcross PR x PREN, where the paternal PR grandparent was female, the colour distribution of the female progeny is virtually identical with that of the PR parents. In the backcross of the reciprocal F_1, PR x ENPR, it is the males that resemble their PR paternal grandparents. In each case the colour distribution of the other sex more closely resembles that of its F_1 counterpart.

When fully pale PR were crossed with grade F (ssp. formosus) of strain RB and the F_1 males backcrossed to pale PR females as before, the sex difference is even more striking (Fig. 4 and Table VIII). There is thus left little doubt that at least one factor involved in the control of abdominal pale scaling in strain PR is sex-linked.

Assuming monofactorial sex-linked inheritance of pa the backcrosses may be set out as follows (crossover classes asterisked) :-

			females		males	
PR x PRRB	$\frac{pa\ m}{pa\ m}$ x $\frac{+\ M}{pa\ m}$	⟶	$\frac{pa\ m}{pa\ m}$	$\frac{+\ m}{pa\ m}^{*}$	$\frac{+\ M}{pa\ m}$	$\frac{pa\ M}{pa\ m}^{*}$
	(pale) (F_1)		(pale)	(F_1 type)		(pale)
PR x RBPR	$\frac{pa\ m}{pa\ m}$ x $\frac{pa\ M}{+\ m}$	⟶	$\frac{+\ m}{pa\ m}$	$\frac{pa\ m}{pa\ m}^{*}$	$\frac{pa\ M}{pa\ m}$	$\frac{+\ M}{pa\ m}^{*}$
	(pale) (F_1)		(F_1 type)	(pale)		(F_1 type)

From this it will be seen that in the PR x PRRB backcross the colour distribution of the male progeny should resemble that of the F_1 males with a deviation to paler if crossovers occur. Similarly, the female distribution should resemble that of PR pa females with a crossover deviation to darker forms. In the PR x RBPR backcross the male distribution should resemble that of PR pa males with darker crossover forms and the females should be like the F_1 females, but include paler crossover forms. The deviations of the observed colour distributions are in all cases in the directions required by crossing over.

As a test for crossovers, grade H males from both PRRB and RBPR backcrosses were mated to grade Q females from the PRRB backcross (parental strain females were not available) which may be assumed to have been homozygous pa. As detailed in Table IX and shown in Fig. 5, the males from the two backcrosses gave identical results, indicating that the grade H males from the RBPR backcross were crossovers, genotypically similar (with respect to pa) to the grade H

males from the other backcross. There would be no such identity were the deviations from the F_1 or parental distributions caused by modifiers or environmental effects. As a further test for crossovers the palest males from backcross PR x PRRB were paired singly with the palest females from backcross PR x RBPR. The most extreme such pair (L4♀ x P1♂) died, the progeny from a J6♀ x J4♂ and a J6♀ x J3♂ are detailed together (being very similar) in Table IX and Fig. 5. The four possible genotypic combinations (crossovers asterisked) with expected progeny are as follows :-

		expected progeny			
i) $\frac{pa\ m}{pa\ m}^{*} \times \frac{pa\ M}{pa\ m}$		progeny as PR-pale abdomen			
ii) $\frac{+\ m}{pa\ m} \times \frac{pa\ M}{pa\ m}^{*}$		$\frac{pa\ m}{pa\ m}$ (pale)	$\frac{+\ m}{pa\ m}$ (F_1 type)	$\frac{pa\ M}{+\ m}$	$\frac{pa\ M}{pa\ m}$ (pale)
iii) $\frac{pa\ m}{pa\ m}^{*} \times \frac{+\ M}{pa\ m}$		progeny as PR x PRRB			
iv) $\frac{+\ m}{pa\ m} \times \frac{+\ M}{pa\ m}$		$\frac{pa\ m}{pa\ m}$ (pale)	$\frac{pa\ m}{+\ m}$ (F_1 type)	$\frac{+\ M}{pa\ m}$	$\frac{+\ M}{+\ m}$ (wild-type)

Matings (i) and (iii) are obviously ruled out, and the distribution in Fig. 5 is clearly close to that indicated by the expected progeny of mating (ii), suggesting that grade J6 in PR x RBPR is not a crossover class while J3 in PR x PRRB is.

Estimation of linkage between pa and M can at most be an approximation, as the identification of crossover classes is somewhat arbitrary. It seems reasonable to take the following limits: PR x PRRB females, darker than grade J4 (13 examples); males, paler than grade H (8 examples); PR x RBPR females, paler than grade J6 (1 example); males, darker than grade J1 (6 examples). This gives a total of 28 suspected crossovers in 523 backcross progeny, and a crossover value of 5.35% between pa and M which would be very close to re (assuming M to be terminal). The constant association of re with colour grade H in the GA line, from which re was first isolated, in the presence of a probable allele of pa (vide infra) further supports the probable close linkage between re and pa.

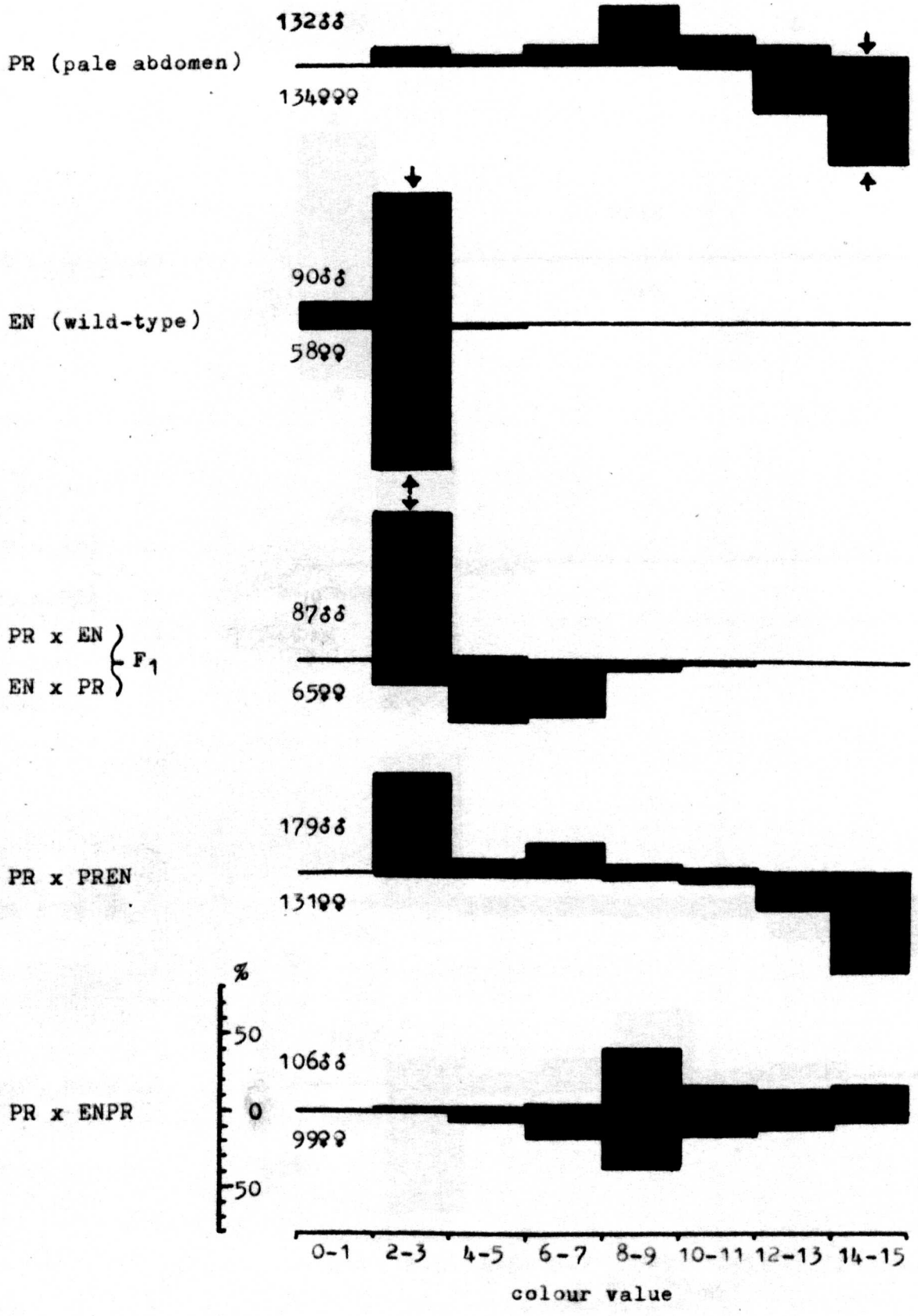

Fig. 3. Distribution according to colour value of PR pa and EN wild-type and the result of crosses and backcrosses. See Fig. 1 for note.

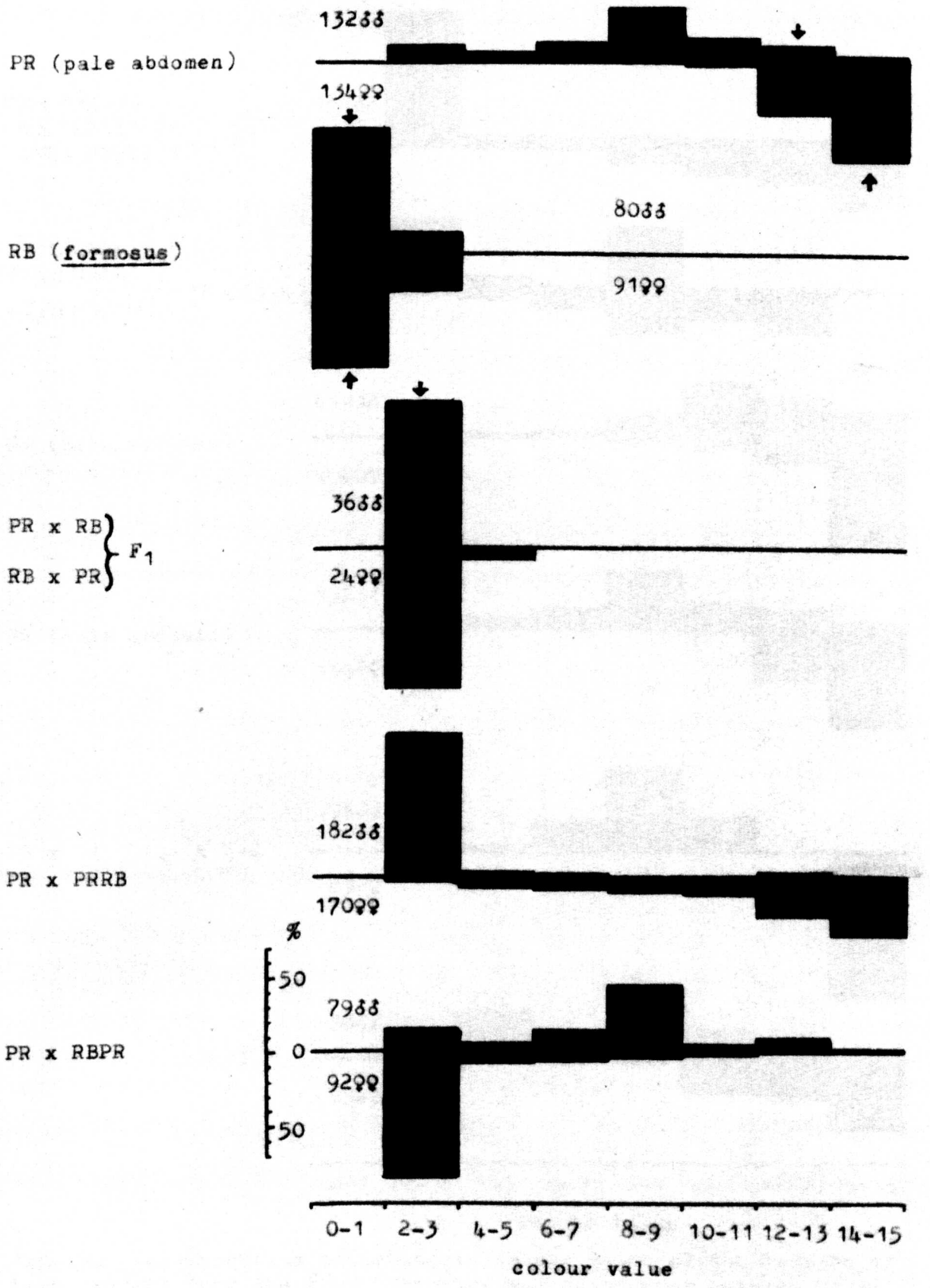

Fig. 4. Distribution according to colour value of PR pa and RB formosus, and the result of crosses and backcrosses. See Fig. 1 note.

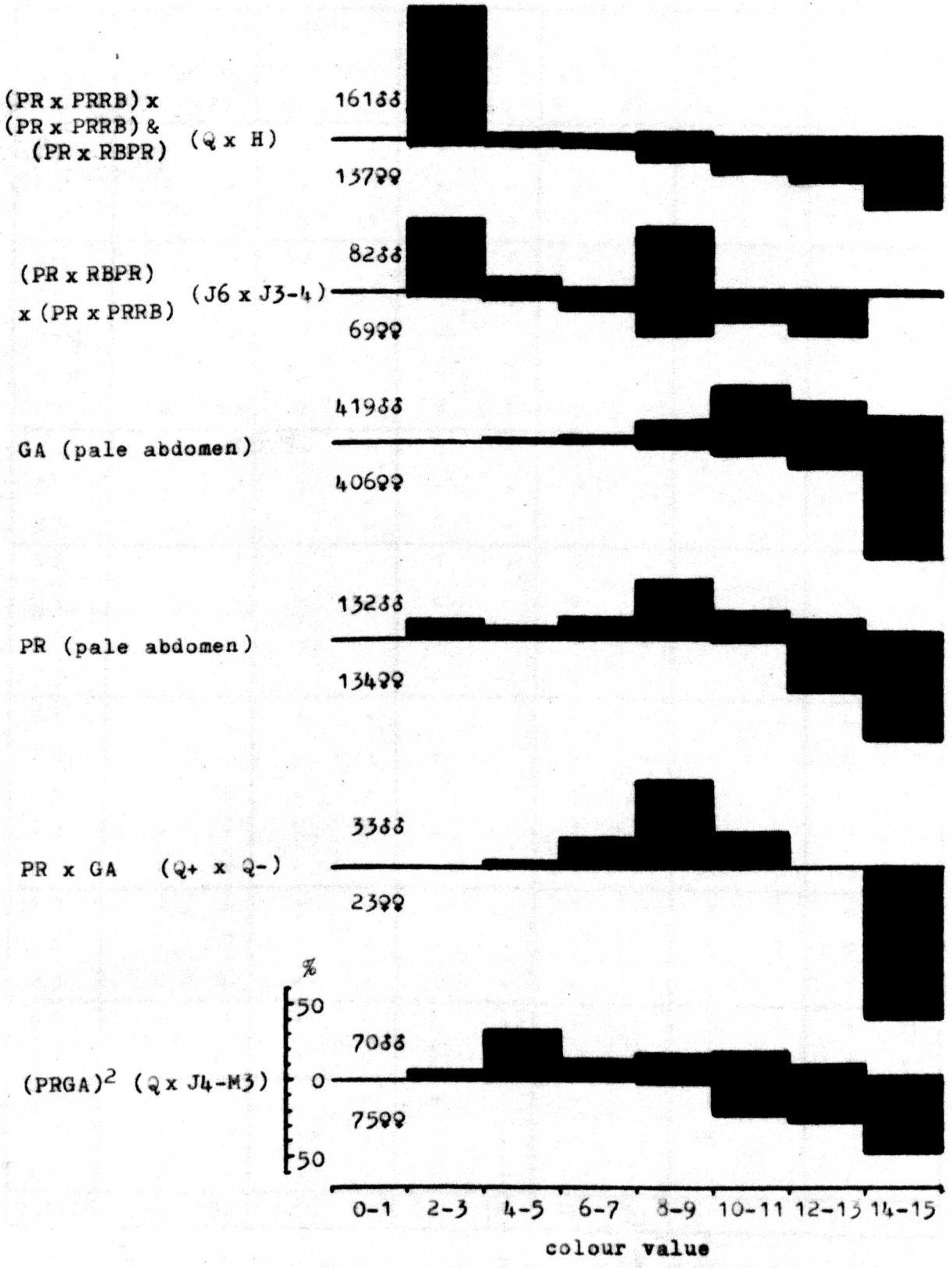

Fig. 5. Distribution according to colour value of the results of test crosses from the Fig. 4 crosses and of crosses between GA pa and PR pa.

TABLE VII

COLOUR GRADE	PR/pa-pale abdomen ♀♀	PR/pa-pale abdomen ♂♂	EN/+ wild-type ♀♀	EN/+ wild-type ♂♂	PR x EN (♀ x H) ♀♀	PR x EN (♀ x H) ♂♂	EN x PR (H x ♀) ♀♀	EN x PR (H x ♀) ♂♂	PR x PREN (♀ x H) ♀♀	PR x PREN (♀ x H) ♂♂	PR x ENPR (♀ x H) ♀♀	PR x ENPR (♀ x H) ♂♂
F	-	-	-	1	-	-	-	-	-	-	-	-
G-	-	-	-	1	-	-	-	-	-	-	-	-
G	-	-	1	11	-	-	-	-	-	-	-	-
H-	-	-	10	40	-	1	-	1	-	7	-	-
H	-	2	21	37	-	44	-	35	-	105	-	1
H-ap	-	-	7	-	-	-	-	-	-	-	-	-
H ap	-	2	15	-	-	2	-	-	-	1	-	-
J 1	-	10	2	-	8	1	2	2	1	2	-	1
J 2	-	5	-	-	4	-	7	1	-	10	2	-
K1	-	-	1	-	-	-	2	-	-	-	-	-
J 3	-	5	1	-	5	-	8	-	1	6	6	2
K 2	-	8	-	-	1	-	7	-	-	-	-	-
J 4	-	3	-	-	4	-	4	-	1	10	8	2
K 3	-	4	-	-	3	-	1	-	-	-	-	-
J 5	-	1	-	-	2	-	-	-	-	26	11	4
L 2	-	-	-	-	-	-	2	-	-	-	-	-
K 4	-	3	-	-	-	-	-	-	-	-	4	1
J 6	-	19	-	-	-	-	-	-	-	8	12	17
L 3	-	-	-	-	1	-	2	-	1	-	1	-
K 5	-	28	-	-	-	-	-	-	3	2	20	25
M 2	-	-	-	-	-	-	1	-	2	-	2	1
L 4	-	7	-	-	-	-	-	-	1	1	7	13
M 3	1	16	-	-	1	-	-	-	5	1	8	5
N 2	1	4	-	-	-	-	-	-	5	-	4	4
P 1	43	10	-	-	-	-	-	-	24	-	8	11
Q-	-	2	-	-	-	-	-	-	-	-	3	15
Q	21	3	-	-	-	-	-	-	13	-	2	3
Q+	68	-	-	-	-	-	-	-	74	-	1	1
TOTALS	134	132	58	90	29	48	36	39	131	179	99	106

TABLE VII. Colour analysis of PR pa and EN wild-type, and the results of crosses and backcrosses. Note - the horizontal lines divide the data into the 8 groups used in the histograms in Fig. 3.

TABLE VIII

COLOUR GRADE	PR/pa-pale abdomen ♀♀	♂♂	RB formosus ♀♀	♂♂	PR x RB (Q+ x F) ♀♀	♂♂	RB x PR (G- x P1) ♀♀	♂♂	PR x PRRB (Q x H) ♀♀	♂♂	PR x RBPR (Q x H) ♀♀	♂♂
F	-	-	18	37	-	-	-	-	-	-	-	-
G-	-	-	20	18	-	-	-	-	-	-	-	-
G	-	-	32	13	-	-	-	-	-	-	-	-
H-	-	-	20	12	-	-	-	2	-	15	1	-
H	-	2	1	-	-	20	6	14	2	159	33	6
H- ap	-	-	-	-	-	-	-	-	-	-	1	-
H ap	-	2	-	-	2	-	10	-	-	-	23	-
J 1	-	10	-	-	4	-	-	-	1	4	18	7
J 2	-	5	-	-	2	-	-	-	4	-	3	2
K 1	-	-	-	-	-	-	-	-	-	-	1	-
J 3	-	5	-	-	-	-	-	-	6	2	3	3
K 2	-	8	-	-	-	-	-	-	-	-	-	1
J 4	-	3	-	-	-	-	-	-	3	1	3	5
K 3	-	4	-	-	-	-	-	-	-	-	-	-
J 5	-	1	-	-	-	-	-	-	10	-	3	6
K 4	-	3	-	-	-	-	-	-	-	-	-	1
J 6	-	19	-	-	-	-	-	-	7	-	2	26
L 3	-	-	-	-	-	-	-	-	1	-	-	-
K 5	-	28	-	-	-	-	-	-	9	-	-	9
L 4	-	7	-	-	-	-	-	-	7	-	1	2
M 3	1	16	-	-	-	-	-	-	11	-	-	2
N 2	1	4	-	-	-	-	-	-	10	-	-	2
P 1	43	10	-	-	-	-	-	-	33	1	-	6
Q-	-	2	-	-	-	-	-	-	2	-	-	1
Q	21	3	-	-	-	-	-	-	20	-	-	-
Q+	68	-	-	-	-	-	-	-	44	-	-	-
TOTALS	134	132	91	80	8	20	16	16	170	182	92	79

TABLE VIII. Colour analysis of PR pa and RB formosus, and the results of crosses and backcrosses. Note - the horizontal lines divide the data into the 8 groups used in the histograms in Fig. 4.

TABLE IX

COLOUR GRADE	PRPRRB (Q+) x PRPRRB (H) ♀♀	♂♂	PRPRRB (Q+) x PRRBPR (H) ♀♀	♂♂	PRRBPR x PRRBPR (J6 x J3-J4) ♀♀	♂♂	PR x GA (Q+ x Q-) ♀♀	♂♂	PRGA F_1 (Q+ x M3) ♀♀	♂♂	PRGA F_1 (Q- x J4) ♀♀	♂♂
H-	-	10	-	4	-	-	-	-	-	-	-	-
H	2	68	-	58	1	25	-	-	-	2	-	-
J 1	-	1	-	1	1	15	-	-	-	3	-	-
J 2	2	5	-	1	3	6	-	-	-	4	-	6
J 3	-	1	1	-	1	2	-	1	-	6	-	6
K 2	-	-	1	-	3	-	-	-	-	-	-	1
J 4	-	1	-	-	1	1	-	3	-	1	-	3
K 3	1	-	-	-	4	-	-	1	-	1	-	-
J 5	2	4	-	-	-	-	-	2	-	3	-	1
K 4	-	-	-	-	-	-	-	2	-	-	-	-
J 6	3	6	4	1	9	31	-	5	-	4	-	7
L 3	-	-	-	-	-	-	-	-	-	-	1	-
K 5	4	-	5	-	11	2	-	12	-	-	1	1
M 2	2	-	-	-	1	-	-	-	-	-	-	-
L 4	4	-	4	-	3	-	-	5	-	1	4	4
M 3	9	-	9	-	10	-	-	2	4	3	9	5
N 2	8	-	5	-	3	-	-	-	5	1	2	-
P 1	14	-	10	-	17	-	-	-	8	3	6	3
Q-	1	-	2	-	-	-	15	-	2	-	6	1
Q	4	-	5	-	1	-	3	-	10	-	7	-
Q+	16	-	19	-	-	-	5	-	8	-	2	-
TOTALS	72	96	65	65	69	82	23	33	37	32	58	38

TABLE IX. Colour analysis of test crosses from the crosses in Table VIII, and crosses between GA pa and PR pa. Note - the horizontal lines divide the data into the 8 groups used in the histograms in Fig. 5.

LINKAGE GROUP II

1. *s* - spot. This has already been described above as an autosomal, recessive, fully penetrant, gene affecting the lateral spots and pale scaling of the abdomen. Spontaneous isolate from many strains.

Description and Inheritance

Allelism of the present *s* material (strain GA) with both that used by McCLELLAND (1960b) and by CRAIG *et al*. (1961) and CRAIG and VANDEHEY (1962) was confirmed by reciprocal cross. (The original Kenya strain was not otherwise used in the present studies. Further data on its inheritance are given below (Table XVI), showing, except when involved with another subviable gene, good single-factor ratios.

Alleles at the *s* locus

The intention is now to demonstrate that there is a series of alleles at the *s* locus, which would account for the great variability of expression of *s*. The effects of the putative alleles, with arbitrary and provisional superscripts are described in Table X. From this it will be seen that, in the females at least, homozygous phenotypes have one character in common, the abdominal venter is almost entirely pale scaled even when the dorsum is only slightly pale (Plate X u,q.). This contrasts with *pa* in which the venter is as dark as that of wild-type although the dorsum is paler (Plate

TABLE X

ALLELE	MALE EXPRESSION	FEMALE EXPRESSION	MEAN COLOUR VALUE OF FEMALES	STRAINS USED
s^+	wild-type	wild-type	(2)	all
s^r	wild-type	silver lateral spots normal, but with antero - posterior extensions of white scales, sternites largely pale-scaled	3.8	DK
s^p	as wild-type except for absence of lateral spots on the seventh segment	as s^r females but pale extensions more like a complete lateral white stripe	from 5.6 to 8.3	FS
s^w	silver lateral spots absent on all segments, some replacement by diffuse white scales, sternites largely pale-scaled	silver lateral spots absent, in their place a variable amount of diffuse pale scaling tending to become aggregated into an oblique stripe, sternites largely pale-scaled	varying from 2.0 to 14.0 in strain GA after outcrossing and selecting for dark or pale	GA BLTS MA KN
s^g	as s^w but lateral white scaling more extensive	as s^w but lateral white scaling almost a contiguous stripe	from 12.3 in EK to 14.2 in AO	AO, DH JD, VL NJ, EK

TABLE X. Descriptions of the four s alleles.

VI a,e,f.). Furthermore, in the females at least, there is always some modification of the lateral spots. All *s* homozygotes show some degree of extra pale scaling on the dorsum, but in contrast to *pa* this is as pale as the basal bands, usually more sharply defined and well developed on the apical margins of the tergites. In crosses to wild-type, the lateral spot modification and ventral paleness are recessive, while the abdominal paleness is semi-dominant; the apical margins of the tergites at least always being noticeably paler in the heterozygotes than in the wild-type homozygotes (*s* homozygotes are illustrated in Plates VIII-X, photographs a-d, g, i-l, n-v and x; the remaining photographs in these plates illustrate heterozygotes).

The different forms of *s* are all allelic because, while in crosses of any one to wild-type the venter and spot effects are always recessive, all crosses between the various forms show the pale venter and at least as much modification of the spots as the least modified parent. The different forms of *s* are not, on the other hand, all caused by the same allele, operating in different genetic backgrounds. If that were so, the F_2 or backcross from crosses between them would not be expected to show clear segregation of the two spot types. For example, DK s^r x FS s^p gave an F_1 like s^r and in the backcross to FS s^p there segregated 14 wild-type (= s^r) : 14 s^p males and 19 s^r : 19 s^p females (actual figures). Table XI shows the various crosses that have been made, all showing allelism.

TABLE XI

ALLELE			s^r	s^p	s^w	s^g
	STRAIN		DK	FS	GA	AO
		MEAN COLOUR VALUE	3.8	5.6	a) 2.6 b) 4.7 c) 7.1	14.2
s^p	FS	5.6	4.6			
s^w	KN	2.3			2.0 (a)	
	BLTS	6.0			4.4 (a)	
	MA	8.1		5.8		
s^g	EK	12.3			5.8 (a)	
	VL	12.3	11.6			
	JD	13.3	11.2		10.1 (b)	
	DH	13.8			12.4 (c)	14.0
	NJ	14.1			11.1 (b)	
	AO	14.2			12.6 (c)	

TABLE XI. Summary of crosses made between the different s alleles and strains.

From the crosses listed in Table XI it is possible to draw some conclusions concerning the dominance hierarchy between alleles. Where the form of the lateral spots is concerned, $\underline{s}^r$ is dominant over $\underline{s}^p$ (as noted above), $\underline{s}^p$ is dominant over $\underline{s}^w$ (although the MA strain of $\underline{s}^w$ was not checked directly with the reference strain GA) and $\underline{s}^w$ is at least partially dominant over $\underline{s}^g$. In all these crosses, the abdominal colour of the offspring was intermediate between that of the parents (figures in Table XI are only given for the females for the sake of clarity).

Here it may be noted that $\underline{s}^g$ is probably allelic if not identical with W (*vide supra*). The strain AO received from Dr. G. B. Craig as homozygous W, was indistinguishable from JD $\underline{s}^g$, and very similar to the other $\underline{s}^g$ strains. Nothing in the description of W by CRAIG and VANDEHEY (1962) is inapplicable to $\underline{s}^g$ and their estimate of crossover between s and y is virtually the same as that between W and y. Because $\underline{s}^g$ and W are so pale, the site of the lateral spots merges with the dorsal paleness (Plate VIII a). The fact that there is no sign of the distinct mirror-like lateral wild-type spots of silver scales was not commented on by Craig and VandeHey. The crosses of strain AO to GA $\underline{s}^w$ (Table XI) gave an F_1 with $\underline{s}^w$-type lateral spots, just as did crosses of $\underline{s}^g$ from other strains to GA $\underline{s}^w$.

A curious feature of the s alleles is that, whereas the effect on the lateral spots is recessive, that on abdominal

paleness is semi-dominant. The different degrees of paleness noted among s alleles may be caused by (a) a pleiotropic effect of the s allele (b) genes at the pa locus (c) genes at other loci.

Although the paleness caused by s^g is much whiter than that of pa homozygotes, control by the pa locus alone would show sex linkage and probably be more recessive. Furthermore, independent assortment could be expected to produce both dark and pale s phenotypes. Fig. 6 and Table XII show in the case of strain DH that there is no change in the colour of s^g homozygotes between the P_1 crossed with wild-type and those segregating in the F_2. This, and the absence of dark s^g forms in populations which are polymorphic for s^g (*vide infra*), indicates that the pale abdominal colour in s^g is mainly a semi-dominant pleiotropic effect of s^g or caused by a closely-linked gene. The fact that the F_1 from the cross JD s^g x GA pa, for example, is as pale as JD, differing only in having silver lateral spots, need not imply allelism with pa, but simply additive interaction between pa and the semi-dominant factor for paleness in the s^g strain.

In contrast to s^g, the colour of the abdominal dorsum of s^w homozygotes is very variable and, as usually isolated, they are darker than pa homozygotes. Starting with s^w phenotypes from strain GA with a mean colour value of about 7 in the males and 8 in the females, selection for pale and dark lines was combined with outcrossing to a grade Q pa s^+ female

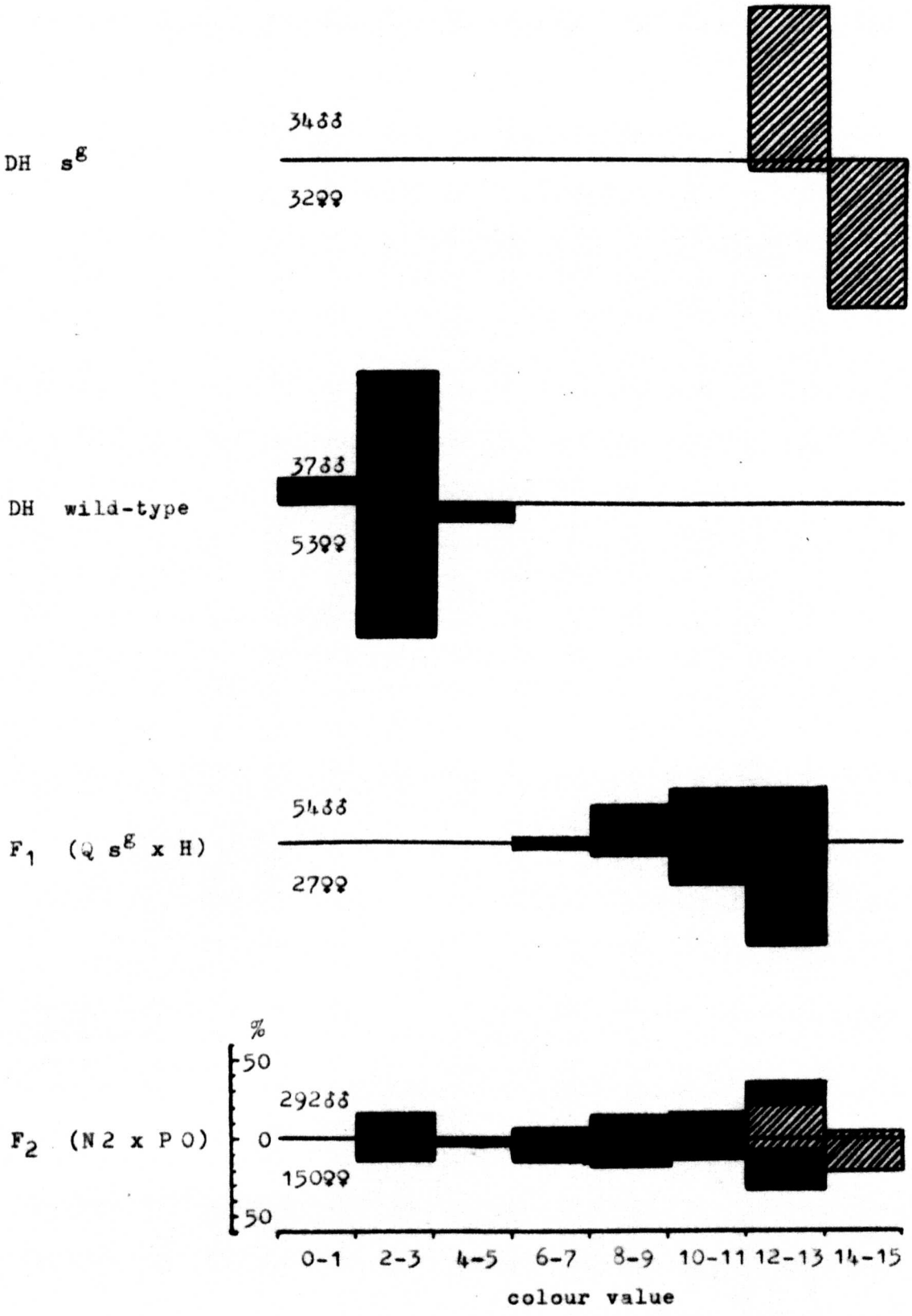

Fig. 6. Distribution according to colour value of DH $\underline{s^g}$, wild-type, F_1 and F_2 crosses. Black = dominant phenotypes, hatched = $\underline{s^g}$.

TABLE XII

COLOUR GRADE	DH $\underline{s^g}$		DH wild-type		F_1 (♀ s^g x H)		F_2 (N2 x PO)			
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀		♂♂	
	all s^g		all s^+		all +		+	s^g	+	s^g
G	-	-	-	6	-	-	-	-	-	-
H-	-	-	-	-	-	-	-	-	5	-
H	-	-	27	30	-	-	11	-	48	-
J 1	-	-	20	1	-	-	6	-	-	-
J 2	-	-	5	-	-	-	4	-	-	-
J 3	-	-	1	-	-	-	1	-	-	-
J 4	-	-	-	-	-	-	3	-	4	-
K 3	-	-	-	-	-	-	1	-	-	-
J 5	-	-	-	-	1	2	5	-	18	-
K 4	-	-	-	-	1	4	6	-	13	-
J 6	-	-	-	-	-	-	3	-	-	-
L 3	-	-	-	-	1	10	11	-	31	-
K 5	-	-	-	-	-	-	8	-	2	-
M 2	-	-	-	-	1	4	6	-	33	-
L 4	-	-	-	-	1	-	8	-	-	-
N 1	-	-	-	-	2	15	2	-	22	-
M 3	-	-	-	-	3	-	-	-	-	-
P 0	-	5	-	-	-	13	-	-	21	5
N 2	-	-	-	-	10	1	9	-	10	-
P 1	2	29	-	-	7	5	27	10	8	61
Q	30	-	-	-	-	-	-	29	-	11
TOTALS	32	34	53	37	27	54	150		292	

TABLE XII. Colour analysis of DH $\underline{s^g}$, wild-type, F_1 and F_2 crosses. Note - the horizontal lines divide the data into the 8 groups used in the histograms in Fig. 6.

(also of strain GA) in the pale line and 4 outcrosses to grade F $\underline{s}^+$ in the dark line (Fig. 7). The pale line died out at a maximum of grade Q^+ females and M3 males, almost the same level of paleness as the pa strains, while the dark line reached an assymptote at grade H- to J1.

The 4 outcrosses to grade F $\underline{s}^+$ gave heterozygotes (shown by downward arrows in Fig. 7) of almost identical colour grade despite the gradual darkening of the line. This suggests that the original material contained a recessive factor (or factors) for paleness that was removed by selection and also a dominant factor which persisted - since the heterozygotes were never as dark as the grade F parent, and ultimately the same colour as the $\underline{s}^w$ homozygotes. This "factor" could plausibly be the pleiotropic paling effect of $\underline{s}^w$. A further outcross to grade F and selection up to the 17th generation gave no reduction in pale scaling, although there was a tendency towards forms lacking basal bands (Plate X x). By this time, the line must have contained virtually the whole grade F genotype with the exception of those genes at and near the s locus. Thus the fully dominant effect of the $\underline{s}^w$ gene (or one closely linked to it) on abdominal colour is sufficient to suppress the expression of the grade F genotype.

There remained a further point to establish. The production of a pale $\underline{s}^w$ form by outcrossing the source material to GA pa and selecting might be due not to the pa

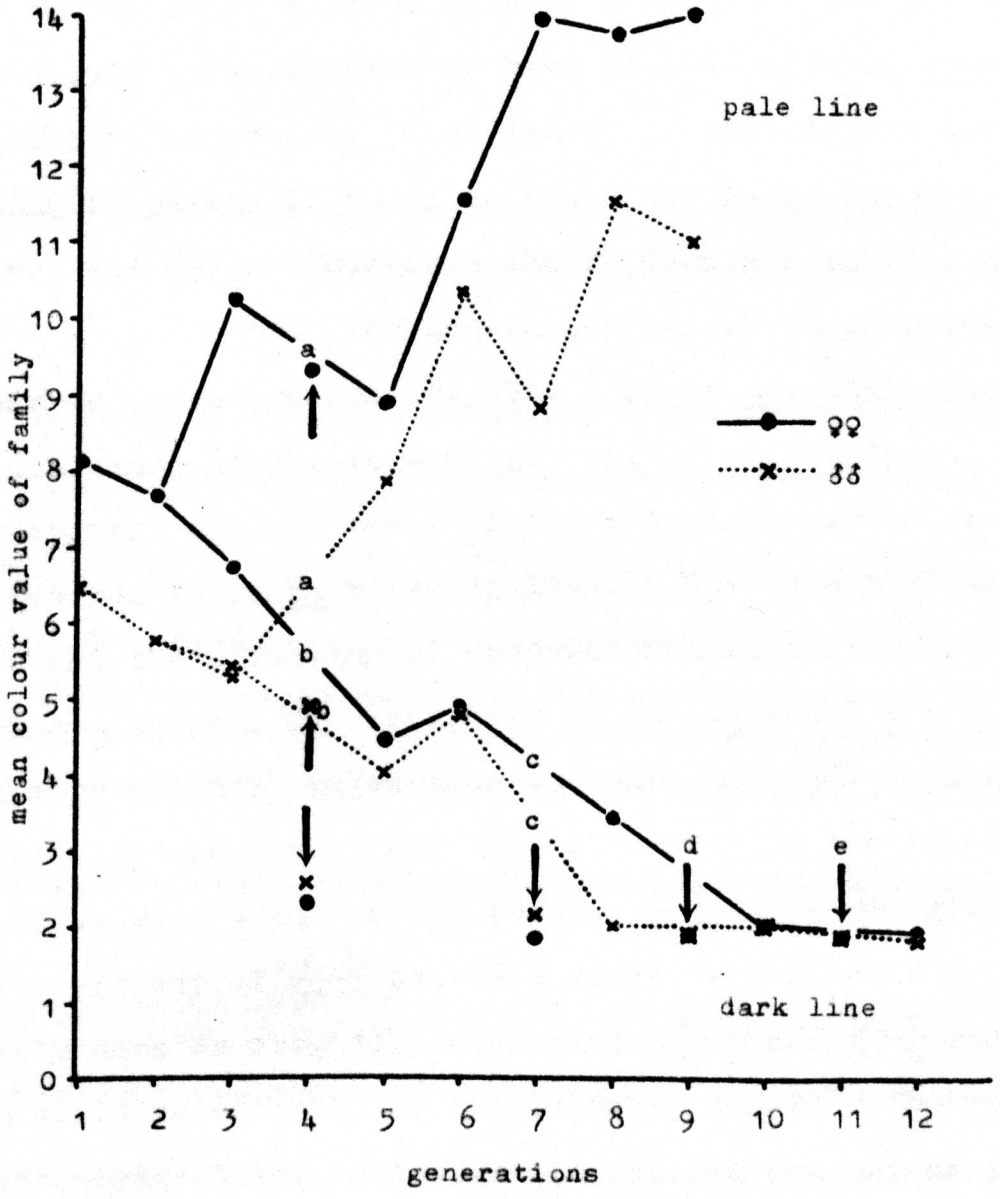

Fig. 7. The results of selection, with outcrossing, for dark and pale $\underline{s^w}$ lines. Note - the arrows point to the heterozygote values, upwards for the pale line and downwards for the dark line.

gene, but to other factors already present in the $\underline{s}^{w}$ strain. Such factors were presumed to have been eliminated from the dark line by generation 16 at least. Crossing and backcrossing to pa was of no value since the backcross progeny would be all $\frac{s^{w}}{s^{+}}$. Therefore the F_1 from the initial cross $\underline{s}^{+}$ pa x $\underline{s}^{w}$ pa^{+} (dark line generation 16) was backcrossed to the dark $\underline{s}^{w}$ line (generation 17). Since pa, while hardly semi-dominant, does have a slight paling effect as a heterozygote, some at least of the palest backcross progeny were assumed to be pa heterozygotes. The palest $\underline{s}^{w}$ were paired and the offspring of one such single pair (Fig. 8) showed a clear colour dimorphism in the females, the males being very slightly paler than the dark line $\underline{s}^{w}$. Since pa is partially sex-linked and was introduced in the female, the last cross was, with respect to pa, $\frac{+\ M}{pa\ m}$ x $\frac{+\ m}{pa\ m}$, giving an F_2 type progeny of $\frac{+\ M}{pa\ m}$ and $\frac{+\ M}{+\ m}$ males, which would be normally dark or slightly paler, and $\frac{+\ m}{pa\ m}$ and $\frac{pa\ m}{pa\ m}$ females, which would be slightly or considerably paler. The results show precisely this; although the females are not as pale as the final generation of the pale $\underline{s}^{w}$ line they are paler than the $\underline{s}^{w}$ source material. This clearly indicates that the effects of pa and $\underline{s}^{w}$ can be combined.

Following a cross of FS $\underline{s}^{p}$ with PR pa the F_2 and subsequent generations were selected for pale $\underline{s}^{p}$ forms. A female from the F_4 is illustrated (Plate X s) showing, in addition to the $\underline{s}^{p}$-type lateral spots, the thin median line

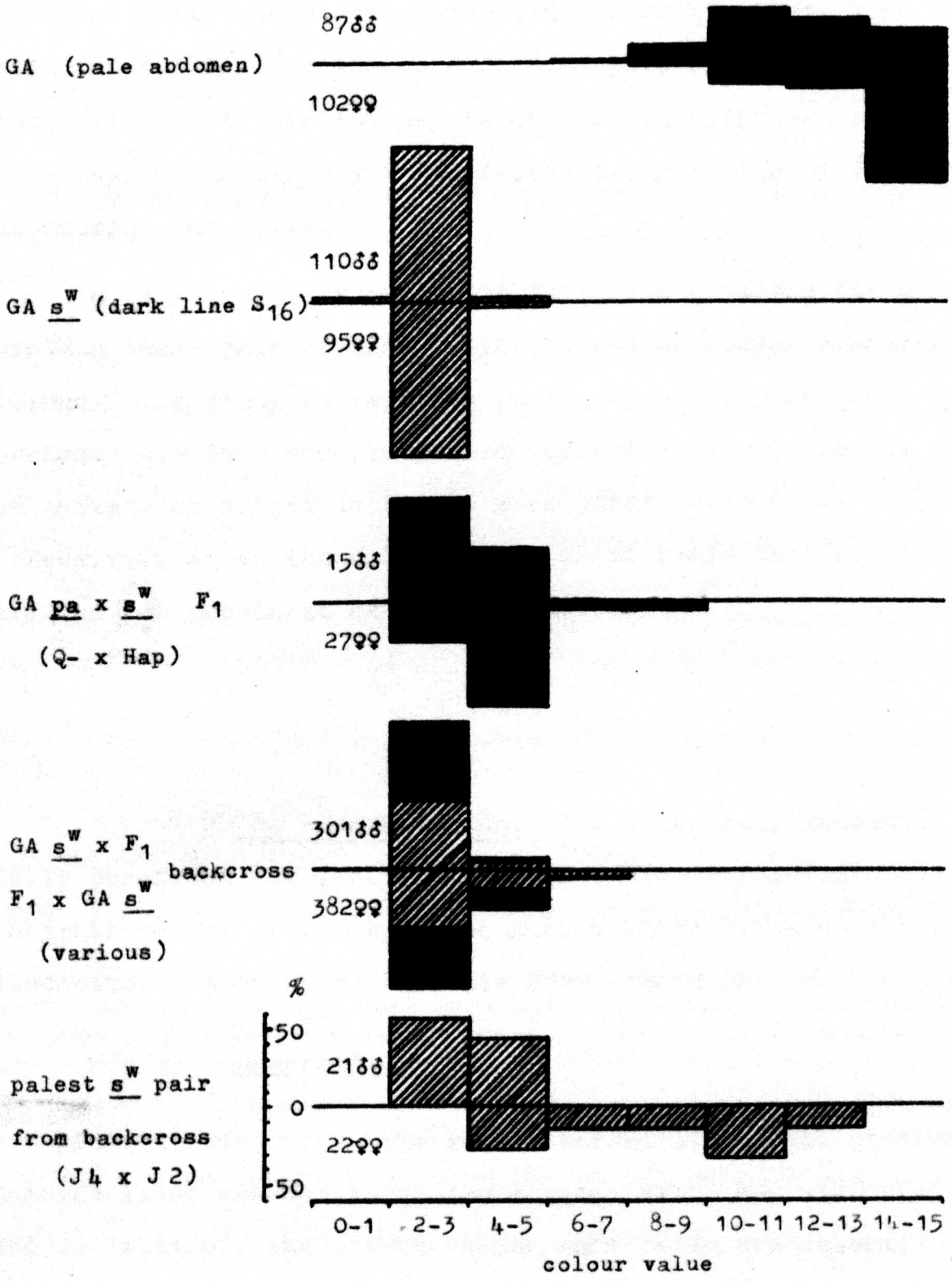

Fig. 8. The distribution according to colour value of GA pa and GA s^w (dark line) and the results of a cross between them, backcrosses to s^w and a selective mating for paleness.
Black = s^+ phenotypes, hatched = s^w phenotypes.

of dense white scales characteristic of FS $\underline{s}^{p}$ (Plate X r) standing out against the more diffuse pale scaling of pa, suggesting that this too may be associated with the gene $\underline{s}^{p}$. DK $\underline{s}^{r}$ was not similarly investigated owing to the difficulty of scoring the males.

In summary, it is suggested that each s allele has a certain semi-dominant pleiotropic effect on dorsal abdominal colour. This may be large as in $\underline{s}^{g}$, in which case the presence of other more recessive factors causing paleness will be masked, or slight as in $\underline{s}^{w}$, when other factors may effectively alter the colour. It is probable that pa or its alleles are prominent among such factors.

2. ds - dark scutum. An autosomal, recessive, fully penetrant gene, of slightly variable expression, controlling the presence of the narrow white lines on the mesonotum. Spontaneous isolate from strain RB.

Description

In the mutant form the median paired lines, the postero-lateral lines and the three small lines at either side of, and in front of, the pre-scutellar bare patch are absent, leaving only the crescent-shaped anterolateral spots visible (Plate XIII a). In the males the lines are consistently

absent and the ground colour of the scutum is densely black, in the females there is often a slight trace of greyish scales at the site of the median paired lines.

Inheritance

In all F_2 and backcross families ds gives clear single factor ratios. The initial isolation occurred in a family also segregating for s^w where the absence of double recessives indicated probable linkage. However, some intermatings of probable s^w heterozygotes, among the ds class, gave the double recessive. In all the double recessives, but in none of the single recessives, the vertex of the head was almost completely black scaled (Plate XIII a). This may be an additive effect of ds and s^w. Two series of backcrosses of double coupling-heterozygotes to double homozygotes (Table XIII) gave 14 single recessive crossovers in a total of 731. This gives a crossover value of 1.92 between ds and s. Values for the two backcross series separately were 0.75 and 5.12.

To test for possible allelism or interaction between G and ds, a line of the KR strain was used which bred true for Gold females (Plate XIII i). The male expression, said to be recessive by CRAIG and VANDEHEY (1962) was not observed - KR G behaved rather as a sex-limited dominant in females, and was possibly not identical with Craig's factor Gold. Crosses of RB - ds males to Gold females of strain KR gave an entirely

TABLE XIII

PHENOTYPE	MALES			FEMALES			TOTALS
	a	b	total	a	b	total	
+ +	58	202	260	44	88	132	392
ds s^w	54	174	228	29	68	97	325
total non crossovers	112	376	488	73	156	229	717
ds +	5	0	5	1	2	3	8
+ s^w	2	2	4	2	0	2	6
total crossovers	7	2	9	3	2	5	14
TOTALS	119	378	497	76	158	234	731

TABLE XIII. Crosses showing linkage between ds and s^w .

wild-type F_1. The F_2 segregated as follows :-

	wild-type	G	ds	Total
males	90(83%)	0	18(17%)	108
females	26(54%)	11(23%)	11(23%)	48

The failure of the dominant effect of G to appear in the F_1 females and the segregation of about 25% instead of the expected 75% in the F_2 suggests that the darkening effect of ds interacts with the paling effect of G. So it is nevertheless possible that G and ds are allelic.

Unless G and s are on either side of y in chromosome II the crossover values given by CRAIG and VANDEHEY (1962) suggest that they are closely linked (vide supra). The close linkage found between ds and s does not therefore conflict with the hypothesis that ds and G might be allelic.

LINKAGE GROUP III

1. blt - black tarsi. As mentioned above, an autosomal recessive, fully penetrant, gene principally affecting the width of the basal white tarsal bands as in var. atritarsus Edw. Homozygous in strain BLTS supplied by G. B. Craig. Spontaneous isolate of probable allele in strain SK, SO, CN, KN, etc.

Description

Each tarsal segment of the wild-type metathoracic leg is pale scaled as follows :-

segment 1, about the basal 1/5-1/4; segment 2, basal 1/4-1/3; segment 3, basal 1/3; segment 4, basal 2/3; segment 5, whole (Plate XII b).

In the blt homozygote the white bands on all segments are reduced to very narrow rings (as in Plate XII d). The depth of banding at the base of tarsal segments 1 and 2 of the pro- and mesothoracic legs is also greatly reduced.

Inheritance

The BLTS strain was also homozygous for s^w and was used to determine the linkage of th (vide infra). Analysis of the F_2 progenies from parental crosses of the type blt s^w x blt^+ s^+ (Table XVI "coupling") and blt^+ s^+ x blt s (Table XVI "repulsion") provides confirmatory evidence of clear 3:1 ratios with no suggestion of sex-linkage, nor of

linkage between blt and s (vide infra).

Alleles of blt

The tarsi in strain BLP are similar to those of strain BLTS, but are associated with a similar reduction in pale scaling of the palps. This does not appear to segregate from black tarsi and might be a pleiotropic effect. The F_1 from a BLTS x BLP cross had black tarsi and normal palps, thus the black tarsi genes in the two strains are allelic if not identical. Phenotypes identical (with respect to tarsi and palps) to those of BLTS were isolated from strain SK (Plate XII d). Tests for allelism have not been made so this type is provisionally termed blt^1. There also occurred in strain SK and many other strains, a variation (provisionally termed blt^2) which differed from the wild-type only in the 4th and 5th segments of the metathoracic legs. These were both pale scaled on the basal halves (Plate XII c). This gives the hind legs the black-tipped appearance, intermediate between blt^1 and wild-type, characteristic of descriptions of var. luciensis Theo. There is a tendency in some individuals for the pale band to be wider on the dorsal surface, giving it an oblique appearance when viewed laterally.

The results of several crosses in strain SK are given in Table XIV from which the following conclusions may be drawn: Cross (i) shows that blt^2 is recessive to wild-type, and crosses (iii) and (iv) that blt^1 is a recessive allele of

TABLE XIV

PARENTAL PHENOTYPES	PROGENY PHENOTYPES			
	blt^1	*blt*^{2}	wild-type	TOTAL
i) wild-type x wild-type (2 families)	0	40	120	160
ii) *blt*^{1} x *blt*^{1}	23	0	0	23
iii) *blt*^{1} x *blt*^{2} (2 families)	49	53	0	102
iv) *blt*^{2} x *blt*^{2} (sibs from iii)	14	37	0	51

TABLE XIV. Results of six single-pair crosses involving *blt* alleles.

blt^2, cross (iii) gives a clear backcross-type ratio indicating that the blt^2 parents were heterozygous for blt^1. The clear 3 : 1 ratio in cross (iv) confirms this.

In a backcross with wild-type heterozygotes, phenotypes resembling blt^2 from strain CN showed a clear 1 : 1 ratio, blt^2 13♀♀, 21♂♂ : wild-type 19 ♀♀, 15 ♂♂ (X^2 = 2.13, P > 0.2). A cross with BLP demonstrated allelism with blt. Hind tarsi of all F_1 progeny were black-tipped. A similar phenotype from strain KN crossed to wild-type TW gave a wild-type F_1 and segregation of 121 wild-type : 39 black-tipped, an almost perfect 3 : 1 ratio.

2. th - hooked hind tarsi. Recessive autosomal gene affecting principally the shape of the 4th and 5th adult metatarsal. Penetrance possibly incomplete, expression slightly variable. Spontaneous isolate from strain GA.

Description

In the normal and th pupa the legs are bent in gentle curves independently of the limb joints. At emergence the legs are pulled out of the curved pupal integument and in the normal mosquito at once straighten out (Plate XII b). In the mutant th the legs straighten with the exception of the 4th and 5th metatarsi which retain the sharp dorsal curve of

the pupa (Plate XII a). KITZMILLER (1958) reports an analogous variant in C. p. fatigans.

Inheritance

Although th bred true as a colony, one rather poorly fertile single-pair mating of th phenotypes produced 4 wild-type : 12 th progeny, implying either impenetrance or that one supposed homozygous recessive parent was a heterozygous phenocopy. F_2 data from 17 families, in 10 highly homogeneous groups (Table XV, $\chi^2 = 5.10$, $n = 9$, $P > 0.8$) total 1136 wild-type : 249 th. which differs greatly from a 3 : 1 ratio and most closely approaches a 13 : 3 ratio ($\chi^2 = 0.543$, $n = 1$, $P > 0.3$, < 0.5). Three explanations may be offered; th is -

1) subvital
2) incompletely penetrant
3) only effective when homozygous in presence of another dominant gene (epistasis).

If epistasis is assumed th phenotypes can be of two sorts depending whether homozygous or heterozygous for the other factor; therefore F_1 genotypes from th phenotype x wild-type will be of two sorts, either possessing the other factor or not, and there would be three possible F_1-type matings, only 1 mating in 4 giving the 13 : 3 ratio, and the mean more than 8 : 1. Since the observed ratio is derived from homogeneous data from approximately 25 F_2 families,

epistasis is ruled out and the resemblance to a 13 : 3 ratio must be fortuitous.

Of the total F_2 data, 3 families (Table XV I) are from the parental cross wild-type x th and comprise 15 th : 107 + females and 29 th : 103 + males, the difference between the sexes is just significant, ($\chi^2 = 4.15$, $n = 1$, $P < 0.05$) indicating possible sex-linkage. In the larger sample of 12 F_2 progenies from the reciprocal cross th x wild-type (Table XV C-H, J), however, the difference between 76 th : 346 + females and 81 th : 392 + males is quite insignificant, ($\chi^2 = 0.12$, $n = 1$, $P > 0.7$). th is therefore autosomal (unless remotely linked to sex).

Of the total F_2 data 5 families (Table XV C-G) were from a trihybrid cross th blt^+ s^+ x th^+ blt s and 7 families (Table XV I,J) were from the coupling version th blt s x th^+ blt^+ s^+. Table XVI gives the observed counts of the 8 possible F_2 phenotypes in the coupling and repulsion crosses.

Comparing the factors in pairs for evidence of linkage χ^2 values are as follows :-

	blt/th	blt/s	s/th
Repulsion χ^2	2.8	0.71	0.09
Coupling χ^2	155.2	0.01	2.72
Total χ^2	158.0	0.72	2.81
n	2	2	2
P		> 0.5	> 0.2

clearly indicating linkage between blt and th.

TABLE XV

ref.	No. of families	PROGENY +	th	total
A	1	84	22	106
B	1	104	26	130
C	1	63	12	75
D	1	59	10	69
E	1	58	17	75
F	1	34	9	43
G	1	44	9	53
H	3	127	22	149
I	3	210	44	254
J	4	353	78	431
TOTALS	17	1136	249	1385

TABLE XV. Single-family F_2 segregations for th.

TABLE XVI

PHENOTYPE	COUPLING ♀♀	♂♂	tot.	REPULSION ♀♀	♂♂	tot.
+ + +	185	184	369	82	71	153
+ + s	51	72	123	19	18	37
+ th +	14	25	39	18	19	37
+ th s	3	2	5	4	7	11
blt + +	22	31	53	17	34	51
blt + s	9	9	18	9	8	17
blt th +	29	32	61	3	4	7
blt th s	8	9	17	2	0	2
TOTALS	321	364	685	154	161	315

TABLE XVI. F_2 segregations for th and blt in coupling and repulsion.

TABLE XVII

PHENOTYPE	♀♀	♂♂	TOTALS
+ +	101	103	204
blt th	59	75	134
blt +	32	38	70
+ th	14	22	36
TOTALS	206	238	444

TABLE XVII. Backcross data for blt and th in coupling.

Available backcross data (all in coupling phase) is given in Table XVII. Percentage of all crossover classes is $\frac{100\ (70 + 36)}{444}\%$ = 23.87%, but, if impenetrance is assumed, a safer estimate is from th classes only, $\frac{100 \times 36}{170}\%$ = 21.18%.

3. fz - fuzzy. An autosomal recessive gene, probably fully penetrant but certainly sub-vital, affecting the whole body. Spontaneous isolate from GA strain.

Description

All the broad adpressed body scales of the wild-type are replaced in the mutant form by loose etoliated scales which tend to project from the body surface giving the whole mosquito a fuzzy or furry appearance (Plate XVI b). The colour of the scales is normal although the finer details of colour pattern are obscured. The scales of the lateral silver spots seem particularly loose and the integument at the site of the spots is often naked. There is high mortality, particularly among females, resulting from failure to emerge completely from the pupal skin, and many that successfully emerge are "cripples". Adults which look normal, apart from the mutant effect, survive well and are fertile.

Inheritance

Although a single pair mating of fz homozygotes was achieved the low viability precluded colonization. The fz line was four times outcrossed to improve viability and add markers for linkage study. Only four scattered families provided adequate backcross and F_2 linkage data, the relationship between these is shown in Fig. 9.

The first putative backcross-type mating of an fz male to a wild-type sib gave 13 fz : 21 + males and 7 fz : 17 + females. There is no indication of sex-linkage and the significant departure of the total from a 1 : 1 ratio ($\chi^2 = 5.59$, $P < 0.02$) may be attributed to pre-metamorphotic mortality, since adults were classified which had failed to emerge. The second backcross-type mating gave 14 fz : 17 + males and 12 fz : 14 + females. The total segregation does not differ significantly from 1 : 1 expectation ($\chi^2 = 0.44$, $P > 0.5$).

The rather poor F_2 data are given in Table XVIII. A χ^2 test for linkage between s and fz gives the insignificant value of 0.31 ($P > 0.5$) suggesting in the absence of sex-linkage that fz is in linkage group III. The absence of any double blt fz recessives among the total F_2 of 381 could mean either linkage or lethality of such genotypes. There is however no evidence of any such deficiency in the observed segregation of blt which is in almost complete agreement with the expected 3 : 1 ratio.

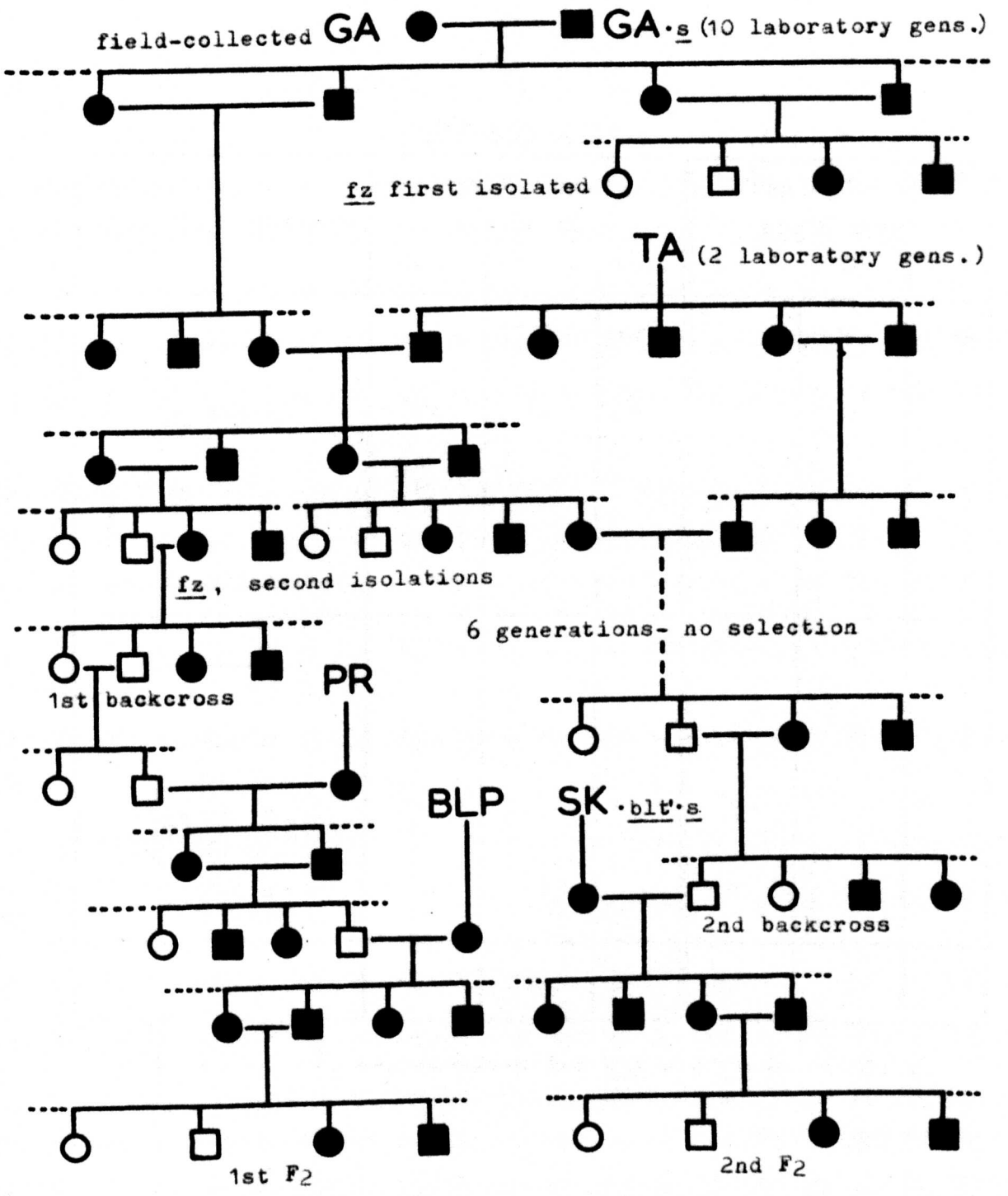

Fig. 9. The "pedigree" of fz.

TABLE XVIII

PROGENY PHENOTYPES	"1st F_2" from blt x fz			"2nd F_2" from blt s x fz		
	♀♀	♂♂	tot.	♀♀	♂♂	tot.
+ + +	110	110	220	3	11	14
+ blt +	40	42	82	3	4	7
fz + +	15	21	36	1	3	4
fz blt +	0	0	0	0	0	0
+ + s	-	-	-	4	5	9
+ blt s	-	-	-	5	2	7
fz + s	-	-	-	1	1	2
fz blt s	-	-	-	0	0	0
TOTALS	165	173	338	17	26	43

TABLE XVIII. F_2 segregations for blt and fz in coupling.

The largest value of the crossover value c consistent with a probability of 0.05 or greater of there being no recombinant phenotypes in an F_2 of 381 animals can be calculated as follows: If $p = \frac{1}{4}c^2$ = frequency of fz blt recombinants then $q = 1 - p$ = frequency of non-recombinants. The probability that, of n animals, all will be non-recombinants is therefore q^n. This gives -

$$q^{381} = 0.05$$

$$\text{so that } \log q = \frac{\log 0.05}{381}$$

$$\text{and } q = 0.9922$$

$$\text{so that } 1 - q = p = \frac{1}{4}c^2 = 0.0078$$

$$c^2 = 0.0312$$

$\therefore$ maximum recombination between blt and fz = c = 0.18

so that the crossover value cannot exceed about 18%. It is more likely to be less.

FACTORS OF UNCERTAIN INHERITANCE

(designations provisional)

1. ol - olive eye. Recessive, autosomal gene of variable expression affecting eye colour. Spontaneous isolate from strain CN.

Description and Inheritance

The dark reddish-green or olive colour distinguishes the eyes of ol from the black wild-type. The hue is however hard to define and cannot be confidently recognized without side-by-side comparison with known or suspected wild-type.

The initial isolation included both sexes, which hinted against sex-linkage. A hand-mated pair bred true but the progeny failed to breed in a paper-cup and the pure ol line was lost. Re-isolation of ol from strain CN has had to be postponed. Females of the original family and later males were outcrossed to re ru double recessives giving a wild-type F_1, indicating no allelism with ru or re. The F_2 from random F_1 pairs segregated to give a bewildering selection of eye colours (Table XIX). Plate V is a photograph of 7 examples from these F_2 progenies. Wild-type, re, ru and ru re were clearly recognized and a 5th class was probably ol (Plate V b,i.), although in the absence of known ol material this could not be absolutely verified. Its virtually equal segregation in males and females confirmed that ol is probably autosomal. Also present were two paler phenotypes

TABLE XIX

PHENOTYPES	F_2 from ol x reru		F_2 from reru x ol		TOTALS
	males	females	males	females	
+ + +	50	70	23	15	158
re + +	21	0	0	5	26
+ ru +	8	5	1	0	14
+ + ol	19	18	6	6	49
re ru +	26	0	0	15	41
re + ol + ru ol } ?	13	12	0	4	29
re ru ol ?	7	0	0	0	7
TOTALS	144	105	30	45	324

TABLE XIX. Segregation of eye colours in F_2 from reciprocal crosses of reru to ol.

(Plate V f,g,m,n.). The palest of these, which in life was a pallid or pinkish grey, was presumed to be the triple recessive homozygote ru re ol. As pupae, some at least of these palest phenotypes showed no eye-pigment whatsoever. Attempts to breed using this class as either parent failed and it is probable that ol, either alone or as the triple recessive, is associated with a behaviour anomaly, perhaps blindness, which prevents mating. At the time of writing intensive steps to re-isolate either ol or the triple recessive have had to be abandoned, but it is noteworthy that continued breeding of the re ru x ol line as a colony consistently produces pupae with unpigmented eyes. None of these however produces adults with eyes resembling the supposed re ru ol homozygote of the F_2. It therefore seems probable that a readjustment of the genotype has occurred in subsequent generations, which increases the rate of red pigment formation in the adult. Future work should be based on pupal eye colour.

2. Probable alleles of pa. Forms with pale abdomens resembling PR pa have been isolated from several strains. Those from strains GA, CC, CR and JA merit special consideration.

Description and Inheritance

GA pa (Plate VI c,d,h.). The palest forms from an initial hatch of wild eggs were mass-mated. Among the progeny, which included many s^w, two single pairs (♀ grade P1 x ♂ K3 and ♀ L2 x ♂ J1) were chosen which were apparently s^+ homozygotes. The palest progeny of these two families (the S_2 generation) were inbred for a generation and then combined. Selective inbreeding was then continued for a further 12 generations, 6 of which were brother-sister matings (Fig. 16). At the end of this period, at the S_{15} generation, no s allele had appeared and abdominal colour. although showing considerable variance, was fairly constant from family to family. The females were as pale and the males a little paler than their PR counterparts. After 7 generations of pale selection there was a slight increase in darker forms, but attempts to apply reverse selection over two generations failed, indicating that this variance was non-genetic. Other indications of possible lack of homeostasis with increasing homozygosity (LERNER, 1954) were pronounced bilateral assymetry (Plate VI c) in later generations compared with the earlier ones or the less inbred strain PR (Plate VI a).

The procedure of testing for sex-linkage by crossing to grade F of strain RB, adopted in strain PR, was repeated. The results are set out in Fig. 10 and Table XX. Despite failure to give as pronounced a demonstration of sex-linkage

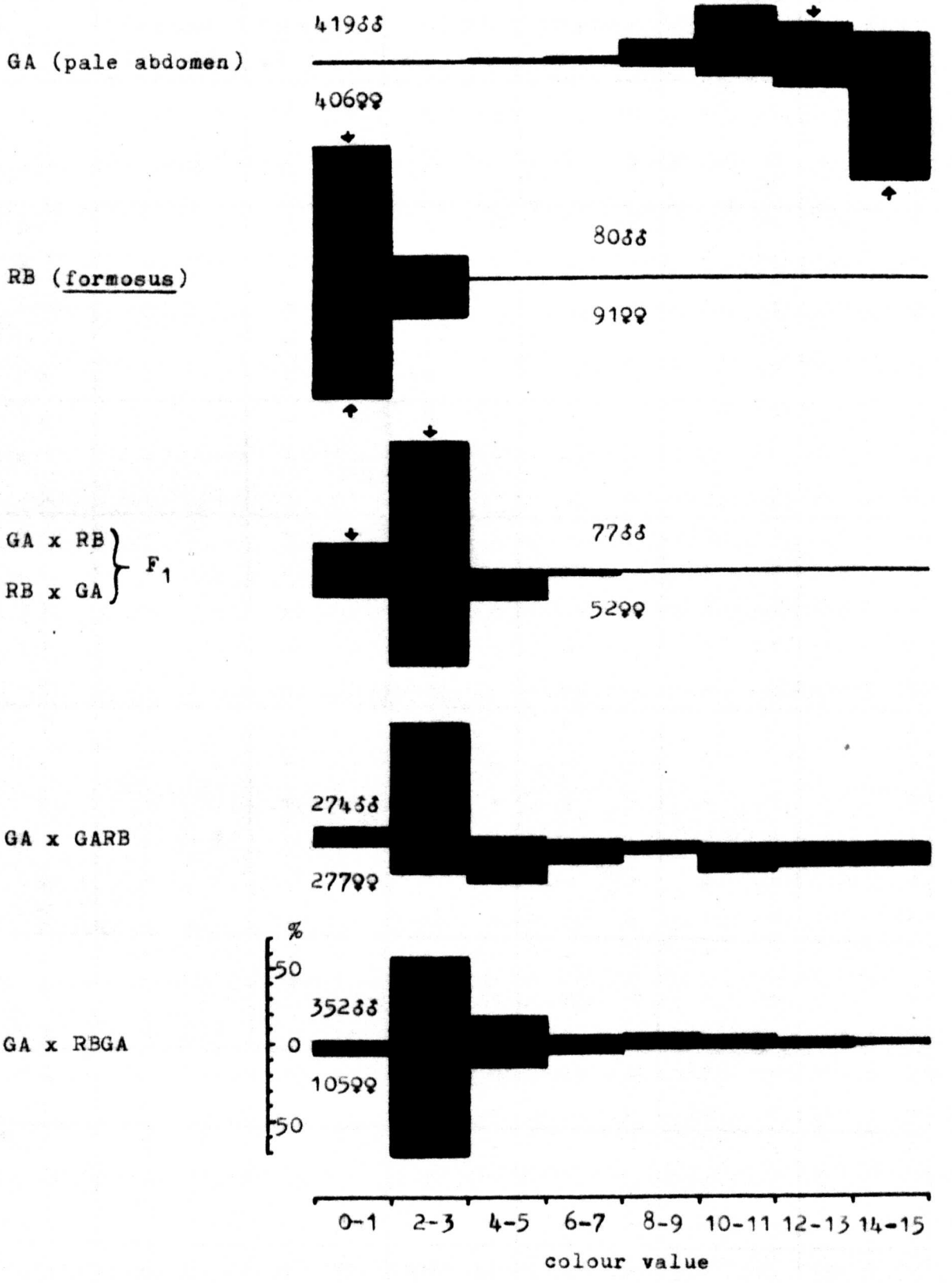

Fig. 10. The distribution according to colour value of GA pa and RB formosus and the result of crosses and backcrosses. See Fig. 1 note.

TABLE XX

COLOUR GRADE	GA/ ? pale abdomen		RB formosus		GA x RB (Q x F)		RB x GA (F x P1)		GA x GARB (Q x F-H)		GA x RBGA (Q x F-H)	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
F	-	-	18	37	-	-	-	2	-	-	-	-
G-	-	-	20	18	-	-	-	4	-	1	-	3
G	-	-	32	13	-	-	9	7	-	28	8	2
H-	-	-	20	12	1	38	9	16	2	96	35	68
H	-	-	1	-	4	8	3	2	6	78	24	87
H- ap	-	-	-	-	-	-	-	-	3	-	-	-
H ap	-	-	-	-	-	-	-	-	4	14	-	1
J 1	-	-	-	-	15	-	-	-	35	31	16	50
J 2	-	-	-	-	8	-	-	-	25	11	4	33
K 1	-	-	-	-	1	-	-	-	2	-	-	-
J 3	-	1	-	-	1	-	-	-	36	7	9	34
K 2	-	-	-	-	1	-	-	-	19	1	2	2
J 4	-	4	-	-	-	-	-	-	1	1	-	9
L 1	-	-	-	-	-	-	-	-	2	-	-	-
K 3	-	4	-	-	-	-	-	-	7	1	4	2
J 5	-	4	-	-	-	-	-	-	-	4	-	6
L 2	-	1	-	-	-	-	-	-	2	-	-	-
K 4	-	7	-	-	-	-	-	-	6	-	-	3
J 6	-	9	-	-	-	-	-	-	-	1	-	8
M 1	-	1	-	-	-	-	-	-	-	-	-	1
L 3	-	2	-	-	-	-	-	-	2	-	-	2
K 5	1	38	-	-	-	-	-	-	1	-	1	11
M 2	-	6	-	-	-	-	-	-	2	-	-	-
L 4	-	51	-	-	-	-	-	-	5	-	-	9
M 3	32	99	-	-	-	-	-	-	43	-	1	15
N 2	27	61	-	-	-	-	-	-	23	-	1	5
P 1	34	49	-	-	-	-	-	-	14	-	-	2
Q-	179	81	-	-	-	-	-	-	16	-	-	1
Q	101	1	-	-	-	-	-	-	21	-	-	-
Q+	32	-	-	-	-	-	-	-	-	-	-	-
TOTALS	406	419	91	80	31	46	21	31	277	274	105	352

TABLE XX. Colour analysis of GA pa and RB formosus, and the results of crosses and backcrosses. Note - the horizontal lines divide the data into the 8 groups used in the histograms in Fig. 10.

as the PRRB crosses, the GARB crosses show evidence of a sex-linked factor. The F_1 included females as pale as grade K1 or K2 (Plate VII k) and showed more variance than the PRRB F_1. This was unexpected in view of the supposedly greater homogeneity of GA. The backcross results resemble those of the PRRB crosses except for the absence of the expected pale males in the GA x RBGA backcross.

The cross between PR and GA (Fig. 5, Table IX) gave an F_1 of mean colour value close to that of PR but with much lower variance, suggesting allelism. Dark and pale selective matings of the F_1 had little differential effect in the F_2. although it is strange that the pale mating produced darker F_2 males than the darker mating.

CC and CR (Plate VII m,p.). Selection for paleness in both these strains (Figs. 17,18.) which, like the PR strain, initially had a few pale females but no males paler than grade H, resulted in lines of pale females and dark males. In the case of CC, crosses of both dark males and pale females to PR pa gave fully pale F_1 females (Plate VII n,o.). There were, however, more partially pale females in the cross PR x CC and more partially pale males in the cross CC x PR (Fig. 11, Table XXI). Assuming paleness to be caused by pa alleles in both strains, it may be supposed that these only occurred in coupling with n in the CC and CR strains as studied.

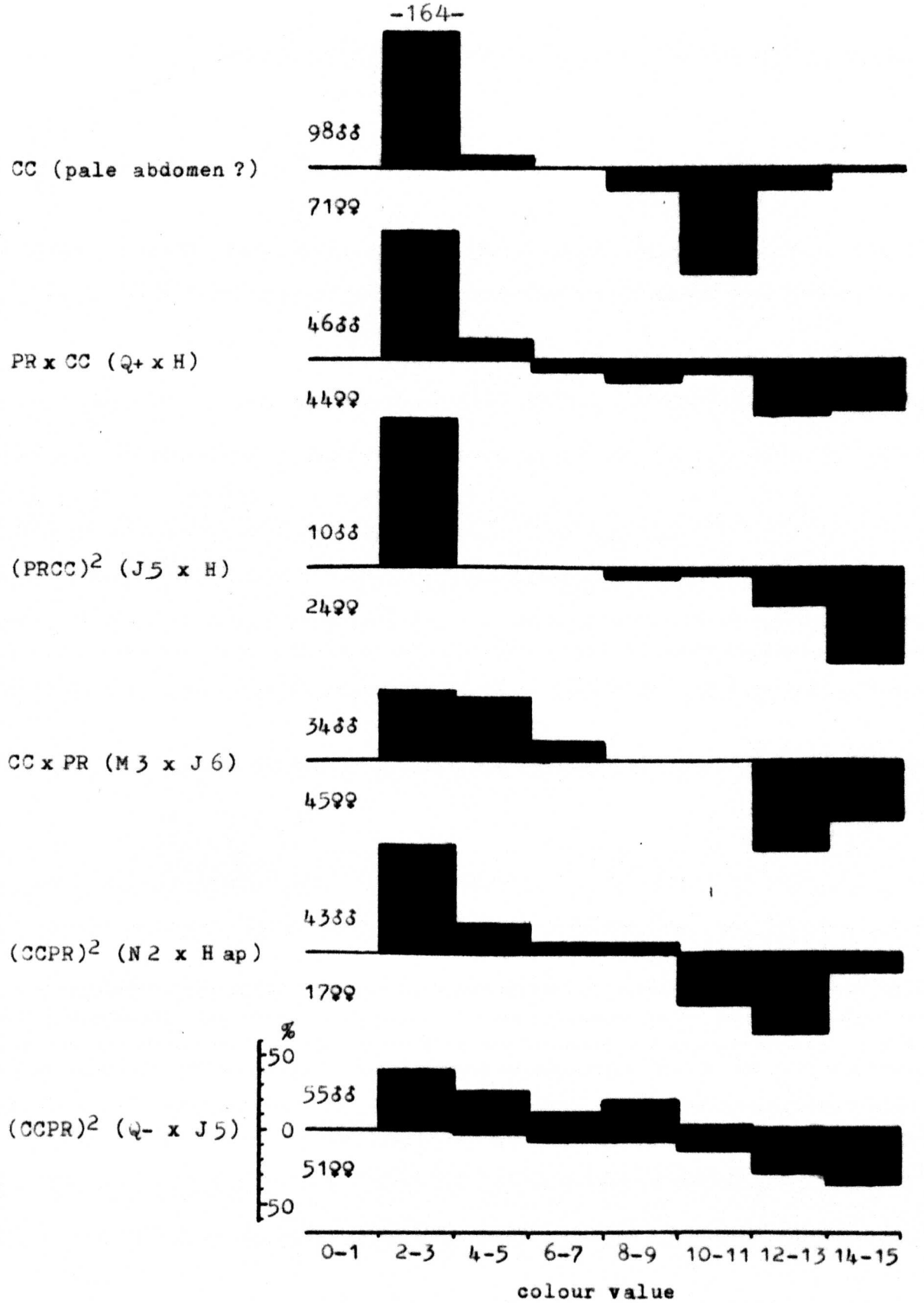

Fig. 11. The distribution according to colour value of CC pa ? and PR pa and the result of crosses between them.

TABLE XXI

COLOUR GRADE	CC/ ? pale abdomen		PR x CC (♀+ x H)		F_2 (J5 x H)		CC x PR (M3xJ6)		F_2 a (N2xHap)		F_2 b (♀- x J5)	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
H-	-	5	-	-	-	4	-	-	-	5	-	7
H	-	12	-	18	-	3	-	1	-	9	-	4
H- ap	-	9	-	20	-	3	-	4	-	1	-	4
H ap	-	46	-	2	-	-	-	4	-	-	-	-
J 1	-	18	-	-	-	-	-	7	-	16	-	7
J 2	-	6	-	-	-	-	-	6	-	4	-	6
J 3	-	2	-	6	-	-	-	8	-	4	1	9
K 2	-	-	-	-	-	-	-	-	-	-	1	1
J 4	-	-	1	-	-	-	-	3	-	1	-	3
K 3	-	-	-	-	-	-	-	-	-	-	4	-
J 5	-	-	3	-	-	-	-	1	-	1	-	2
L 2	-	-	-	-	-	-	-	-	-	-	1	-
K 4	2	-	-	-	-	-	-	-	-	1	1	1
J 6	-	-	4	-	-	-	-	-	-	1	-	10
L 3	-	-	-	-	-	-	-	-	-	-	1	-
K 5	8	-	2	-	2	-	-	-	-	-	2	-
L 4	9	-	1	-	-	-	-	-	-	-	1	-
M 3	41	-	3	-	1	-	-	-	6	-	6	1
N 2	9	-	5	-	2	-	5	-	3	-	4	-
P 1	1	-	11	-	4	-	22	-	6	-	11	-
♀-	1	-	4	-	2	-	17	-	1	-	6	-
♀	-	-	7	-	9	-	1	-	-	-	12	-
♀+	-	-	3	-	4	-	-	-	1	-	-	-
TOTALS	71	98	44	46	24	10	45	34	17	43	51	55

TABLE XXI. Colour analysis of CC pa?and PR pa and the results of crosses between them.

Designating the PR allele as $\underline{pa}^1$ and the CC as $\underline{pa}^2$ the crosses may be represented as follows :-

$$\frac{pa^1\ m}{pa^1\ m} \times \frac{+\ M}{pa^2\ m} \longrightarrow \frac{pa^1\ m}{pa^2\ m} \qquad \frac{+\ M}{pa^1\ m}$$

(fully pale PR) (dark CC) (pale daughters) (dark sons)

and the reciprocal cross

$$\frac{pa^2\ m}{pa^2\ m} \times \frac{pa^1\ M}{pa^1\ m} \longrightarrow \frac{pa^2\ m}{pa^1\ m} \qquad \frac{pa^1\ M}{pa^2\ m}$$

(pale CC) (pale PR) (pale daughters) (pale sons)

The absence of very pale males from the reciprocal F_1 could be because the parents, grade M3 and J6, were far from the palest (the paler pairs having failed to give progeny), or perhaps because $\frac{pa^1}{pa^2}$ is darker than $\frac{pa^1}{pa^1}$. A dark selective mating of the CCPR F_1 produced an F_2 differing from the F_1 only in slightly higher variance. The alternative pale selective mating produced not only a paler F_2 but also one showing much greater variance. Thus, paradoxically, darker females were obtained after selecting for paler (Fig. 11, Table XXI).

One point is however certain, CC males contained one $\underline{pa}$ allele, otherwise there would have been no fully pale F_1 females in the cross to PR. A pale CR female x pale GA male gave a result similar to CC x PR (Plate VII 1). If the hypothesis that paleness in the CC and CR strains is caused by alleles of $\underline{pa}$ is correct, it might be argued that crossing-over would have given pale males. The apparent absence of

such crossovers in these strains may be accounted for in two ways: (i) crossovers are rarer than suggested by the PRRB backcrosses, or (ii) the pa allele is sex-limited just as s^r is among the s alleles.

JA. In the case of JA, isolated early in the work, no tests for allelism with other strains were made, but selection for paleness, carried on until the line died out (Fig. 17) resulted in paler males than in CC or CR. An alternative line of selection in the JA strain is set out in Figs. 12 and 13; the darkest males were paired with the palest females. This resulted in a slight shift to darker in the males and stabilization of the females at about half intermediately pale and half dark. This is most simply explained as follows :-

$$\frac{pa^{?}\ m}{+\ m} \times \frac{+\ M}{+\ m} \longrightarrow \frac{pa^{?}\ m}{+\ m} \quad \frac{+\ m}{+\ m} \quad \frac{+\ M}{pa^{?}\ m} \quad \frac{+\ M}{+\ m}$$

(semi-pale ♀) (darkest ♂) (semi-pale ♀) (dark ♀) (dark ♂) (darkest ♂)

This seems to imply that $\frac{pa^{?}\ m}{+\ m}$ is paler than analogous heterozygotes in strain PR and consequently that $pa^{?}$ either is a different allele of pa or is in a different genetic background. In other strains, selection resulted in an increase in paleness in the females alone, but not to the degree of that in CC or CR (vide infra Figs. 18-20). It is possible that these represent different pa alleles just as

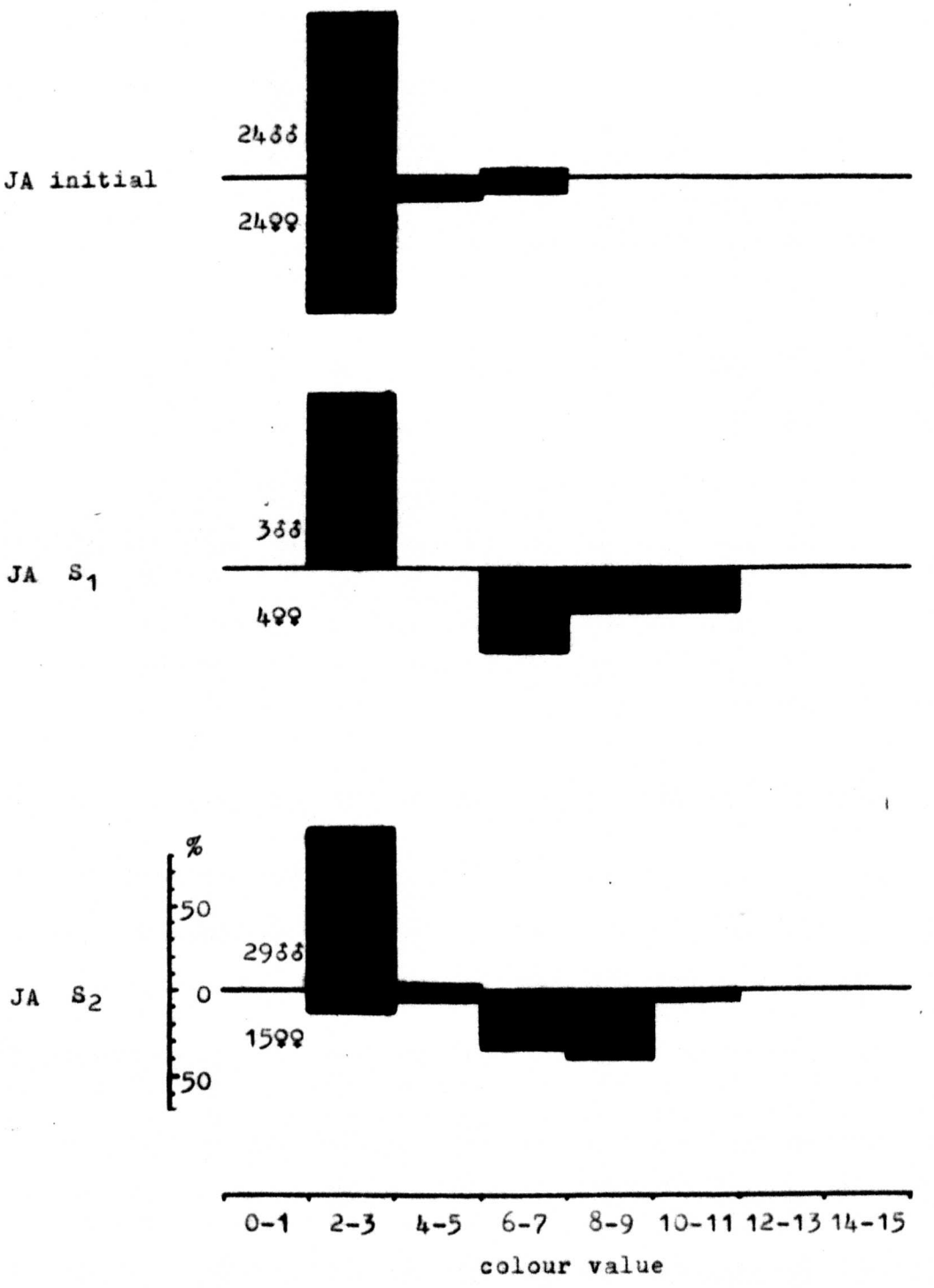

Fig. 12. The distribution according to colour value of JA and the result of selecting palest females and darkest males.

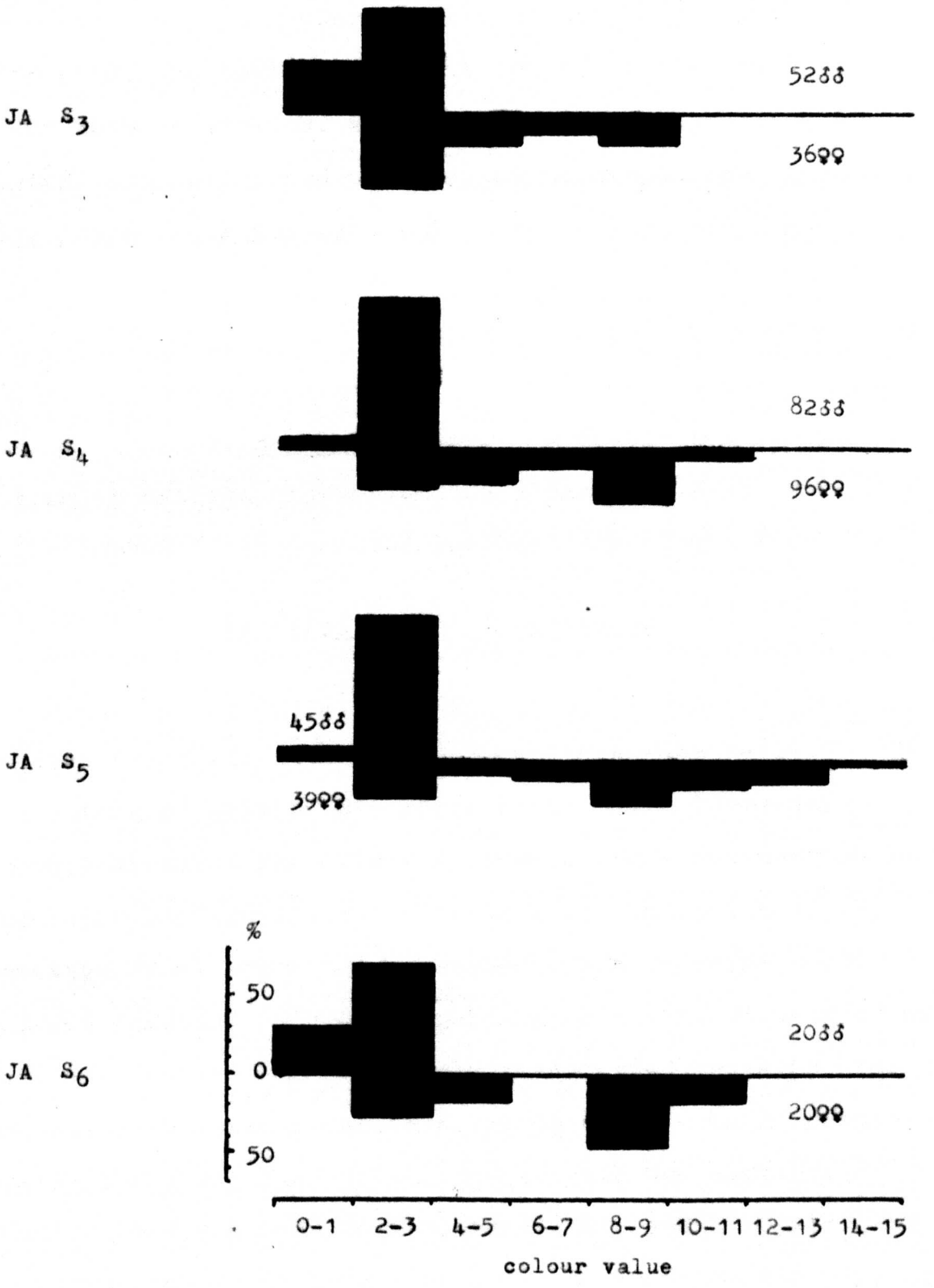

Fig. 13. As Fig. 12, continued for a further 4 generations of selection.

there seem to be a number of alleles at the s locus. On the other hand, the possibility that complex differences in colour between strains, aside from the effects of the s alleles, are influenced by genes at more than one locus (vide infra "formosus abdomen").

3. Basal-bandless. Probably polygenic, causing in extreme expression the absence of basal pale bands on the dorsum. Spontaneous isolate from strain GH.

Description and Inheritance

Black scales speckling the pale basal bands of the tergites frequently occur and appear to answer to the description of bri-brindle reported by CRAIG (personal communication). The offspring from a cross between two such black-speckled individuals, from the initial hatch of GH, consisted of 14 normal : 17 speckled males, 4 normal : 30 speckled females. In 6 of the females the basal band of at least one tergite was completely interrupted medially, rather suggesting the appearance in A. albopictus. In a further 3 females the basal band of the 3rd tergite was completely absent. A cross between a speckled male and a female with an interrupted basal band yielded an F_2 in which all were at least speckled. Of 14 males the basal band of the 3rd tergite was interrupted in 8 and absent in 2. Of 18 females

the 3rd tergal basal band of 5 had 11-15 pale scales, of 3 6-10, of 2 less than 5 or no pale scales. An F_3 from a pair which both lacked basal pale bands on the 3rd tergite was even darker. The number of pale scales in all basal bands was very reduced. An F_4 obtained from still darker parents than those of the F_3 produced a few individuals almost entirely devoid of any pale scales at the site of the normal pale basal bands (Plate 11 h), resembling those described by CONNAL (1927). The 4 generations of brother-sister selective mating resulted in low fertility, no F_5 was obtained from the darkest pairs, other pairs producing an F_5 paler than the F_4. For the same reason outcrosses to the extreme dark $\underline{s}^{w}$ (Plate X x), designed to test for allelism, failed.

4. Possible alleles of ds - other dark scutum variants. Probably recessive, autosomal genes of apparently constant expression, affecting the median lines of the mesonotum. Spontaneous isolates from strains YD and CN.

Description and Inheritance of first variant (strain YD)

The posterolateral lines of the mesonotum are normal but the median paired lines are absent as in ds. The line in front of the prescutellar bare space is greatly exaggerated and those at either side much reduced (Plate XIII c). The

initial segregation was 4 wild-type : 5 mutant males, 3 wild-type : 3 mutant females. One of the mutant females mated with a wild-type male of a related line gave offspring as follows: 19 wild-type : 4 mutant males, 13 wild-type : 11 mutant females. Both these families could have been backcross types, so no conclusion as to dominance is possible. The apparent absence of this mutant in the generation prior to its isolation suggests that it is recessive, in which case sex-linkage would be contra-indicated. Tests for allelism with ds were not made.

Description and Inheritance of second variant (strain CN)

This variant seems to answer to the description of an Australian variety by F. H. TAYLOR (1914). The postero-lateral lines of the mesonotum are normal but the median paired lines and those surrounding the prescutellar bare space are absent (Plate XIII b). Expression is less pronounced in the males, some of the lines being faintly visible. All 11 female progeny of a mutant female and faintly marked male were like the mother, the median lines were absent in 9 out of 16 males and faint in 7. It was not possible to arrange tests for allelism with either ds or the previous variant.

5. Fl - Fleck. Dominant, probably autosomal gene affecting the posteromedian line on the mesonotum. Spontaneous isolate from strain GH.

Description and Inheritance

The line in front of the prescutellar bare space is enlarged into a narrowly elliptical spot, the other lines on the mesonotum are not affected (Plate XIII g). The initial isolation gave 5 wild-type : 7 Fl males, 6 wild-type : 3 Fl females. A male Fl was outcrossed to a female with wild-type thorax. All male progeny were Fl; the pattern in the only 2 F_1 females was indistinct.

6. St - Stripe. Dominant, probably autosomal gene affecting the median paired lines of the mesonotum. Spontaneous isolate from strain PR.

Description and Inheritance

The median paired lines, but none of the other lines, are very broad and conspicuous (Plate XIII f). This is always associated with pa in strain PR but on outcrosses is completely dominant, whereas pa (vide supra) is virtually recessive and is probably caused by a separate linked factor.

7. "formosus" abdomen. The fact that nearly all the A. aegypti encountered away from the vicinity of houses in sub-Saharan Africa lack any pale scaling on the first abdominal tergite led MATTINGLY (1957), as discussed earlier, to separate such a form as a geographical subspecies. Thus this variation is of obvious significance, yet it has defied analysis in the present study. Mattingly's criteria of absence of any pale scales on the first tergite (i.e. colour grade F, Plate XI e,f.) is undoubtedly too stringent, since attempts to select a true breeding strain in which both males and females were grade F always failed. At least some females in every family always had one or more pale scales on the 1st tergite. The same was true when large numbers of bush-collected adults were examined (McCLELLAND 1960 b).

If subspecies formosus is to be regarded as a bionomic entity, it is obvious that it must be re-defined to include the range of colour expression, particularly in the females, up to grade G. On the other hand it is well nigh impossible to define it as a genetic entity on the basis of colour alone (it may well be that other attributes such as behaviour are necessary), since populations show complete intergrading with wild-type and the difference between grade F and wild-type - grade H is by any measure small.

In order to gain some information on the possible mode of inheritance, grade F examples from strain YD were crossed reciprocally with grade H wild-type from strain EN.

Although there was considerable variance among the F_1 progeny, several were unquestionably as dark as the YD parents. Backcrosses of grade F males of the F_1 to grade H females of the EN strain, again produced a number (mostly males) of grade F (Fig. 14, Table XXII).

Comparison between the backcrosses of the heterozygous males from the reciprocal F_1 offspring gives no indication of sex-linkage. In other respects the differences between grades H and F seem clearly polygenic. The genes concerned might well be the modifiers responsible for variance in paler forms.

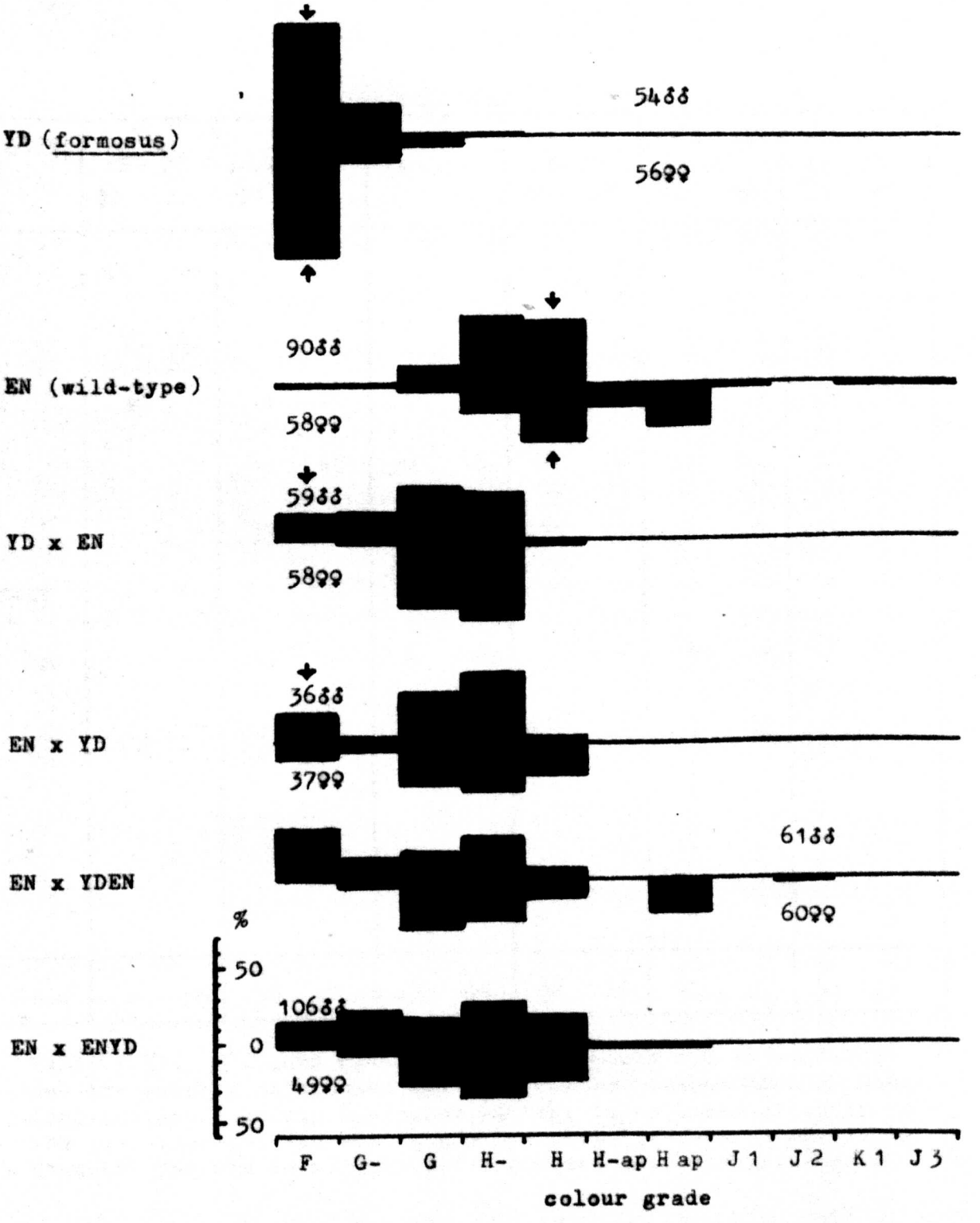

Fig. 14. The distribution according to colour grade of YD formosus and EN wild-type and the results of crosses and backcrosses between them. See Fig. 1 note.

TABLE XXII

COLOUR GRADE	YD (F x F) ♀♀	♂♂	EN (colony) ♀♀	♂♂	YD x EN (F x H) ♀♀	♂♂	EN x YD (H x F) ♀♀	♂♂	EN x YDEN (H x F) ♀♀	♂♂	EN x ENYD (H x F) ♀♀	♂♂
F	44	41	-	1	-	10	4	7	-	20	1	16
G-	9	11	-	1	1	11	2	1	3	9	4	23
G	3	1	1	11	25	20	11	11	19	11	14	18
H-	-	1	10	40	30	18	12	16	17	17	17	28
H	-	-	21	37	2	-	8	1	7	4	11	21
H-ap	-	-	7	-	-	-	-	-	-	-	1	-
H ap	-	-	15	-	-	-	-	-	13	-	1	-
J 1	-	-	2	-	-	-	-	-	-	-	-	-
J 2	-	-	-	-	-	-	-	-	1	-	-	-
K 1	-	-	1	-	-	-	-	-	-	-	-	-
J 3	-	-	1	-	-	-	-	-	-	-	-	-
TOTALS	56	54	58	90	58	59	37	36	60	61	49	106

TABLE XXII. Colour analysis of YD formosus and EN wild-type and the results of crosses and backcrosses between them. Note that the accompanying histogram in Fig. 14 is based directly on the colour grades and not on grouped colour values. Also note that the parents in all the above crosses were either H or F.

OTHER VARIATION

1. Eye colour. Most remarkable are the instances of bluish eyes. These were not irridescent effects but obvious pigment changes. A family derived from a single wild-caught female, strain KN, included 4 females and 1 male with blue eyes, and one male with a single blue eye. Single individuals also occurred with blue eyes in strains DC, DK and PR, magenta-coloured eyes in strain DC, ultramarine eyes in strain GA and eyes with green patches in strain CR. The incidence of several in the single family of KN suggested a genetic cause; however, outcrossing of these to wild-type, followed by three generations inbreeding, failed to effect a re-isolation.

2. Colour of vertex. The line of pale scales on either side of the median cranial sulcus of the head, the narrow line of pale scales bordering the eyes and lateral lines enclose areas behind each eye which can vary from being entirely black-scaled (Plate XIII b,c,d), to being completely white (Plate XIII h). Variation in the median pale line itself was discussed above under ds. Beyond the noting of this variation in most strains it was not studied.

3. Drooping antennae. Recurved, as against straight, male antennae with the hairs adpressed instead of erect were noticed in two strains. Probably corresponding to dr of VANDEHEY and CRAIG (1962).

4. Tufted antennae. A single male of strain VL showed compression or fusion of antennal segments into the length of about two normal segments. The number of hairs was about normal so that each antenna resembled a tuft of hairs sprouting from the torus. This male was caged with, but failed to fertilize, two females.

5. Clubbed palps. The palps of males in strain BLTS sometimes appeared shortened and clubbed, probably the same as kn - knobbed of VandeHey and Craig. Following an outcross, matings of the all-normal F_1 gave an almost perfect 3 : 1 ratio in the F_2 indicating a recessive factor. A variant with warts on the 2nd palpal segment which segregated in a family of the re line was probably wa - wart of VandeHey and Craig.

6. Two-banded female palps. A variant showing a spot of white scales on the normally dark third segment of the female palp occurred in several strains but was not studied. This is presumably the same as sp - speck of CRAIG and VANDEHEY (1962).

7. Bent proboscis. A ventral bend in the tip of the female proboscis was an effectively lethal character since it prevented blood-feeding. This occurred in a number of strains, and in half the females of a single family near the end of the JA pale female x dark male selection line (vide supra). This indicated a backcross of a homozygous male to heterozygous female, the factor being recessive. In another strain of mixed origins, the probosci of several females in a single family showed a "z-bend" (Plate XIX q). It is not known which, if either, of these variants corresponds to hk - hook proboscis of VANDEHEY and CRAIG (1962), since this is only described as having a hook or curve in the middle.

8. Wing variants. Since no careful search was made it is not surprising that little variation was noted in the wings. Three obvious abnormalities (Fig. 15) were noticed, corresponding probably to N - Notch (Fig. 15 b), nt -

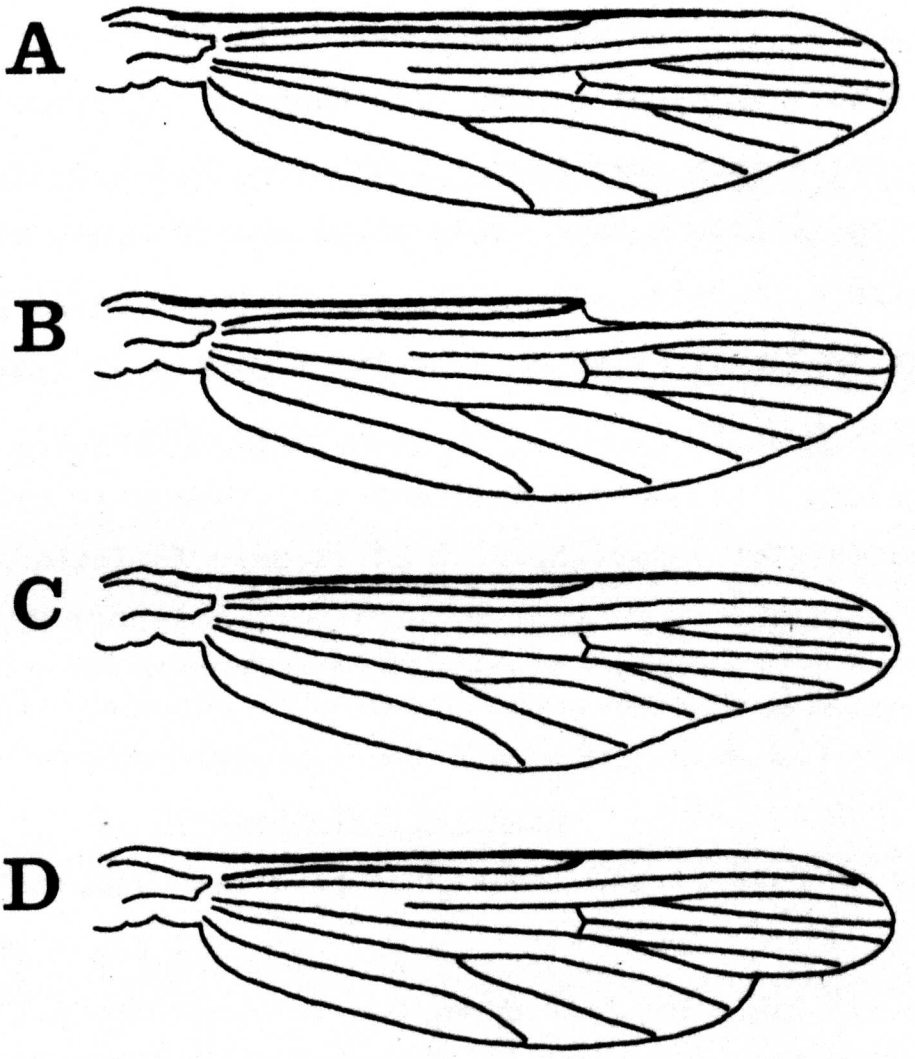

Fig. 15. Sketches of three wing variants, B—D, compared with wild-type, A.

notch trail (Fig. 15 d) and lb - lobe (Fig. 15 c) of VANDEHEY and CRAIG (1962). On the other hand neither the F_1 nor the F_2 from an outcross of supposed N showed the character indicating a probable phenocopy. Down-bent wing tips (c.f. bt - bent of VANDEHEY and CRAIG (1962) and a similar variant in C. p. fatigans (KITZMILLER, 1958) were noticed in strain PR. A female of strain GA with no anomaly other than the right wing 1/5 shorter than the left, was probably a partial gynandromorph or mosaic, the short wing being male (vide infra). A parallel example in C. p. fatigans is also noted by KITZMILLER (1958).

9. Black-scaled halteres. This variant, h of Craig and VandeHey, was frequent especially in strains of colour grade F and G.

10. Possible further blt alleles. In addition to those discussed above a variant with the distal third of the 5th metatarsal black but with the 4th as wild-type, was observed in strain CR. Another variant with the dorsal surface of the metatarsi as wild-type and the ventral as blt^2 appeared in strain MM.

A more remarkable variant appeared in strain RB in which the dorsal surfaces of the tarsi were wild-type and the ventral surfaces as in *blt*^{1} homozygotes (Plate XII e). Provisionally named half-black it is an exaggeration of the tendency noted in *blt*^{2}. All F_1 offspring of a sib with wild-type tarsi x BLP were wild-type. However, the variant reappeared in the offspring of this F_1 backcrossed to wild-type RB. The BLP x RB F_1 must therefore have been doubly heterozygous for *blt* and half-black, indicating that half-black may not be an allele at the *blt* locus. There was no opportunity to apply a direct test of allelism.

11. *Pale prothoracic femurs*. Variants with considerably more pale-scaling than usual on the dorsal surface of the prothoracic femur were characteristic of females of several strains, particularly DK, where its isolation, together with *sr*, suggested linkage. It is of uncertain homology with *li* - lightfoot of CRAIG and VANDEHEY (1962) which was sex-limited to males.

12. *Wide basal bands*. A striking extension of all the basal bands to the lateral edges of the tergites, and their fusion with the lateral spots occurred in a single female of strain GH. Attempted outcrossing was unsuccessful.

13.Abdominal warts. Protuberances of the intersegmental membrane of the abdomen which displaced the tergites were frequent in certain families, particularly GA and PR (c.f. CRAIG et al., 1961).

14.Unilateral development of a tergite. The 3rd or 4th tergite sometimes failed to develop unilaterally resulting in a bent abdomen as in females of strains RB (Plate XVI c) and VZ. In an example from strain JA two "half-tergites" on one side were in apposition to a single tergite on the other side. This may be homologous with twisted or split abdomen mentioned by CRAIG et al. (1961). A rather similar deformity was described by McCLELLAND (1960 b) and BURGESS (1955) noted, in hybrids of A. punctipennis x A. freeborni, some larvae with wedge-shaped abdominal segments. These gave the larvae an angular appearance and suggest that similar deformities probably occurred in the larvae which gave rise to the present adults.

15.Abnormal genitalia. One basimere of a male from strain NJ possessed a strange distal process (Plate XIX p). This may represent a unilateral distortion of the clasper which characterizes hf - half genitalia of VandeHey and Craig.

16. Somatic mosaic. An F_1 female from the cross PR x EN which was heterozygous for pa showed a distinct patch of white scales on the 4th tergite (Plate XVI a). This is presumably a mosaic caused by either a somatic mutation of $pa^+ \longrightarrow$ pa or the loss of a portion of the chromosome carrying the pa^+ allele.

17. Ether sensitivity. A large proportion of a single family of strain YD apparently showed abnormal sensitivity to ether, failing to recover from the normal immobilizing dose.

18. Abnormal sex-ratio. Wide variation in sex-ratio occurred in some strains. This was probably caused by the same or similar factor as Mp of CRAIG et al. (1960). Gross departures from a 1 : 1 ratio were mostly in the direction of excess males and tended to recur in consecutive generations, but successive generations of strain FS produced ratios varying from 10♂♂ : 49 ♀♀ to 22♂♂ : 12♀♀. One family of TW produced no females, and the sex ratios in two F_2 families from an outcross TW x KN were 100♂♂ : 11♀♀ and 44♂♂ : 6♀♀.

COLOUR VARIATION IN THE DIFFERENT STRAINS

PALENESS CAUSED BY FACTORS OTHER THAN s ALLELES

The results of selection for paleness in strains PR, GA, JA, CR and CC have been mentioned earlier and shown in Figs. 1, 2 and 16-18, as probably under the control of closely similar alleles. In the case of GA, Fig. 16 illustrates the distinctly different composition of populations in houses, compared with those from the bush and plantation. The response to selection in strains SG, CN, MI, HW and KR (Figs. 18-20) was of a much lower order and resembled JA, CR and CC in the absence of any real increase in paleness in the males above wild-type grade H. The pale scaling in MI (Plate XI b) and HW was at most very sparse and diffuse, whereas the paler individuals among the other strains showed a broader development of white scales on the second tergite from almost medially pale in SG (Plate XI d) to full width medial paling as in GA or PR, but the more posterior tergites were brindled only. The expression in the palest individuals was quite constant between families of the same strain, but clearly different between the different strains, suggesting qualitative as well as quantitative genetic differences.

Other strains, from which no s alleles were isolated, either showed no initial variation above grade J1 or almost no response to pale-selection other than a lowering in the

frequency of grades F and G, resulting in almost pure wild-type populations.

Histograms (Figs. 21-24) are therefore given for the initial sample only in the case of these strains, TV, SA, DC, TR, BK*, SV*, TW, PS, EO, WL*, SN*, PN*, TN*, TA*, SO* and NR*, and it should be noticed that the horizontal scale is double that normally used, the colour values are not grouped in twos. More than half the strains (asterisked) were freshly field-collected. Some of the paler strains, PR, CR, CC, JA and MI were from laboratory colonies established for several years, so there seems little correlation between laboratory adaptation and colour "potential". It is clear however that initial variance, in the females at least, is highly indicative of the result of selection. The histograms in Figs. 1 and 16-20 show that strains giving the greatest response to pale selection had generally a higher initial frequency of pale forms.

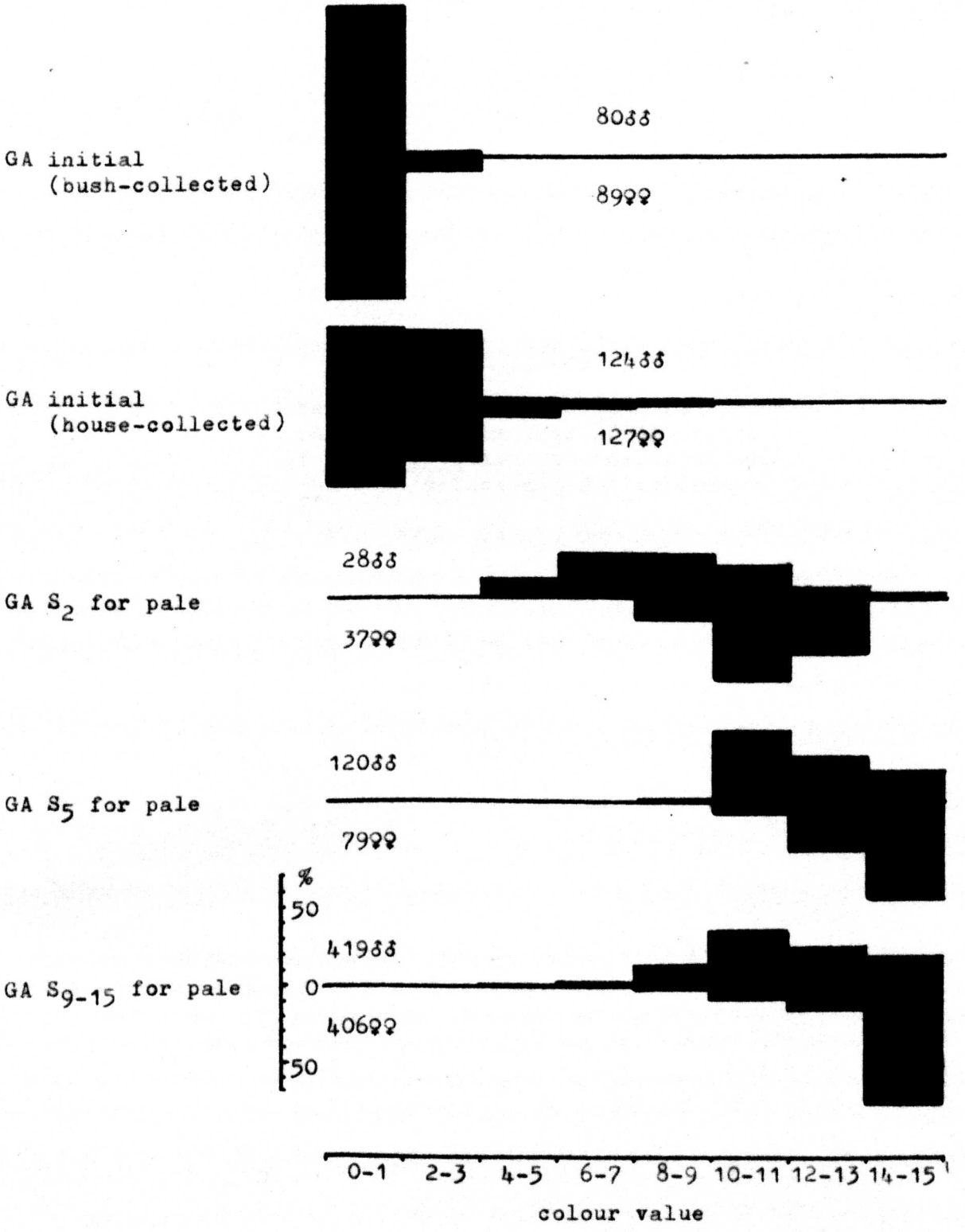

Fig. 16. Distribution according to colour value in a feral population of A. aegypti compared with a domestic population and the result of 15 generations of selection for paleness.

Figs. No. 17-20

The distribution according to colour value of populations of 8 strains of A. aegypti and the result of one or more selections for paleness.

Fig. 17. JA and CR

18. CC and SG

19. CN and MI

20. HW and KR

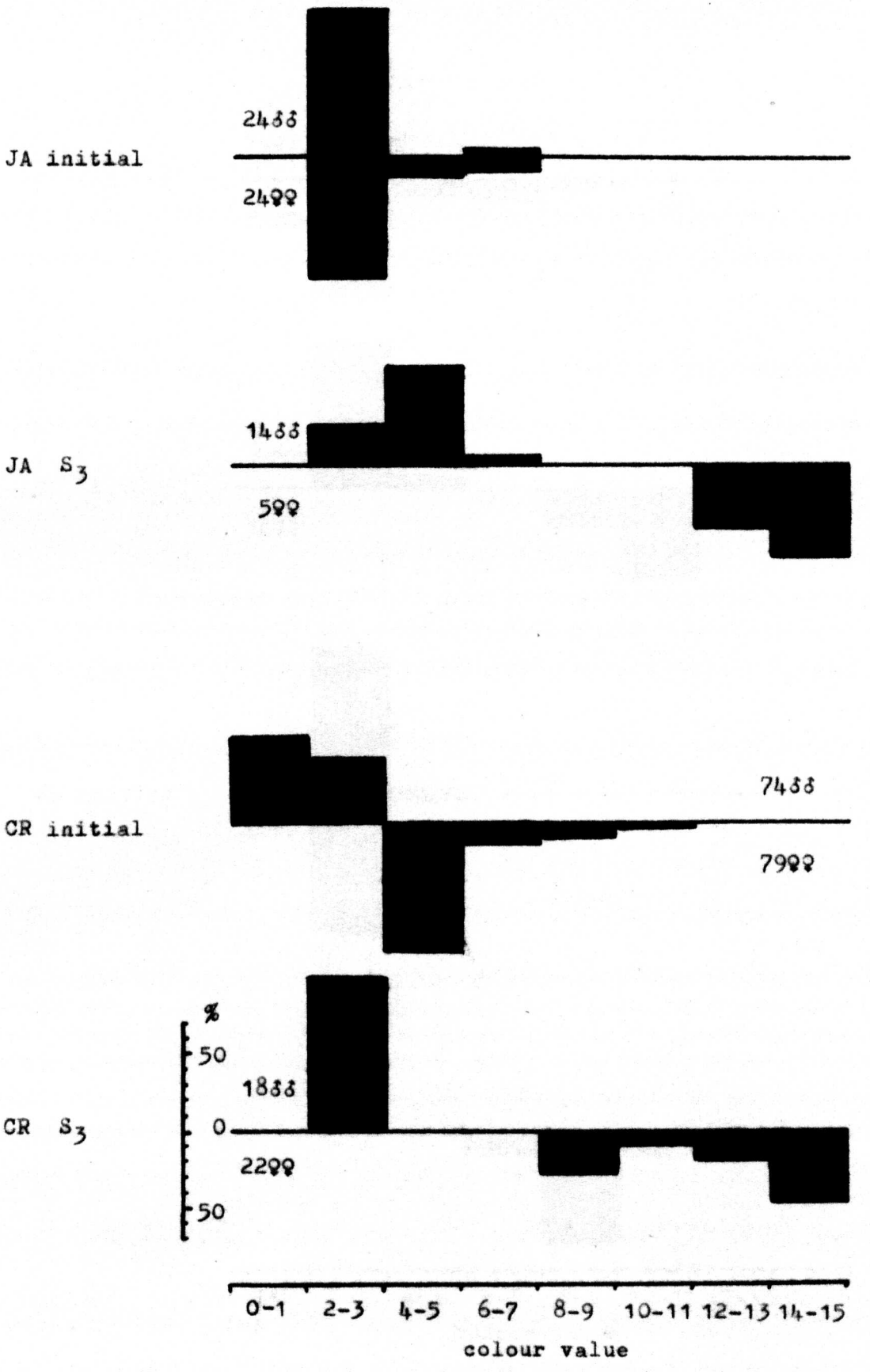
JA initial
24♂♂
24♀♀
JA S_3
14♂♂
5♀♀
CR initial
74♂♂
79♀♀
CR S_3
%
50
18♂♂
0
22♀♀
50
0-1
2-3
4-5
6-7
8-9
10-11
12-13
14-15
colour value

Fig. 17.

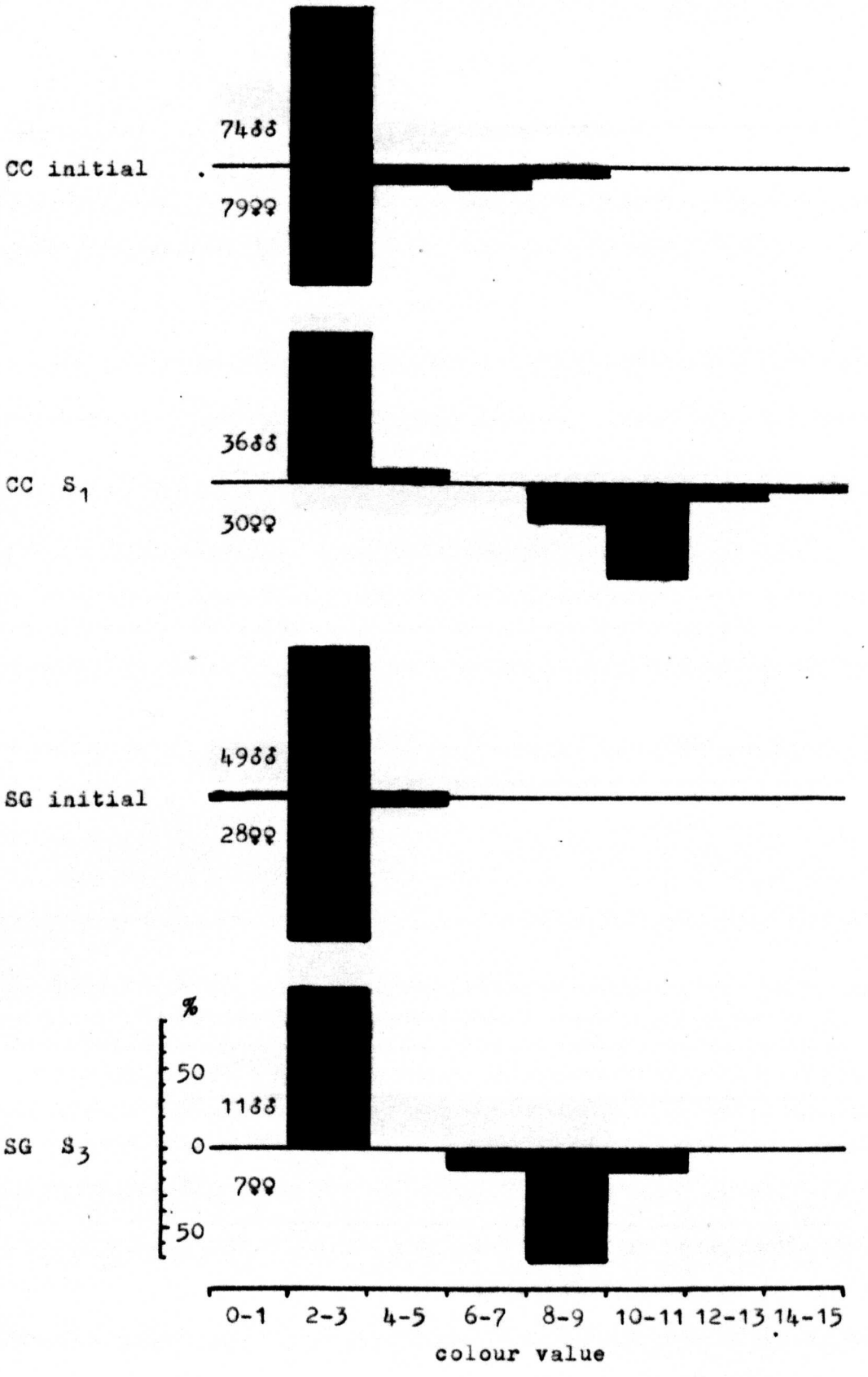
CC initial
7488
7999
CC S1
3688
3099
SG initial
4988
2899
SG S3
%
50
1188
0
799
50
0-1
2-3
4-5
6-7
8-9
10-11
12-13
14-15
colour value

Fig. 18.

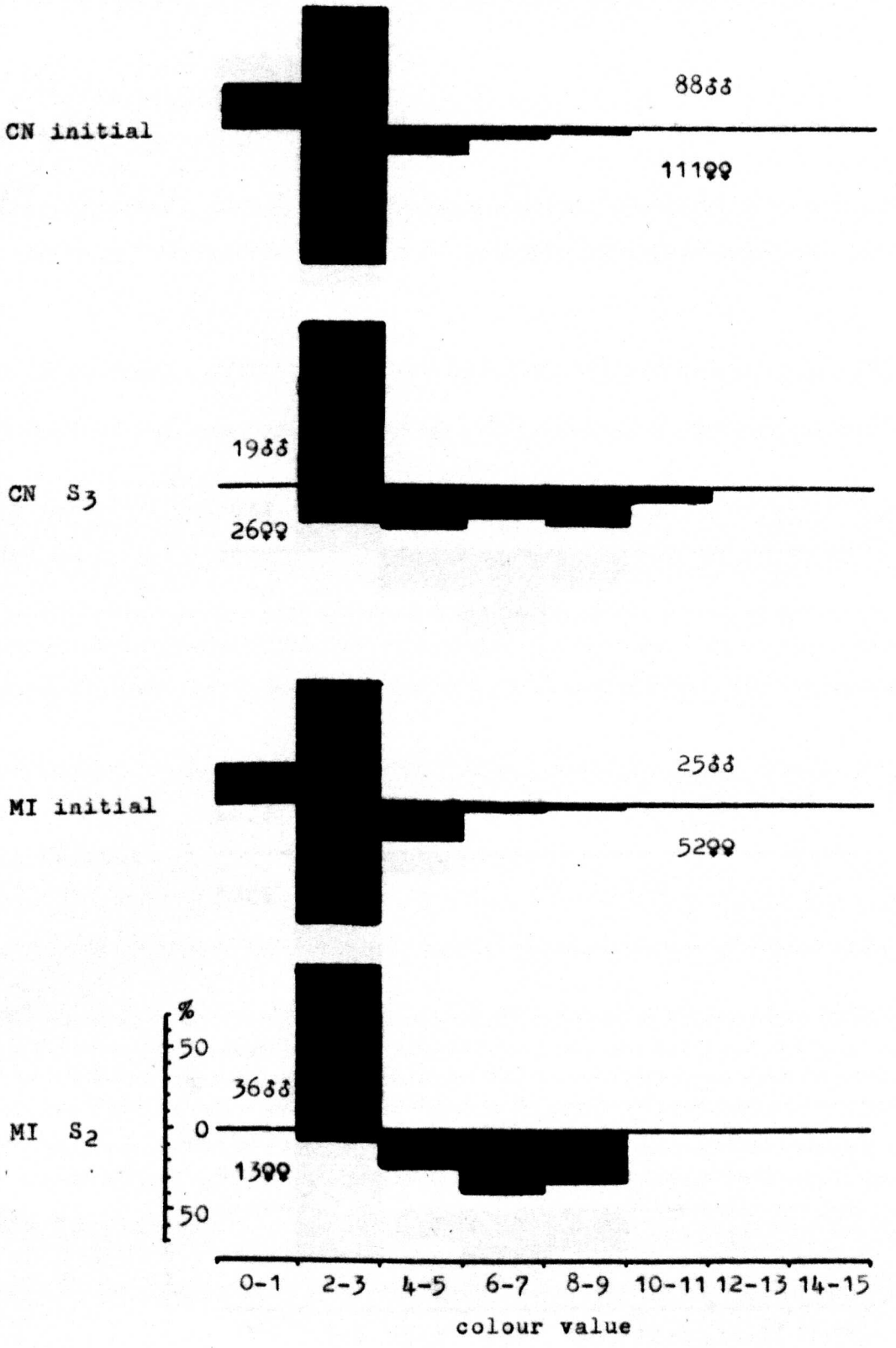
CN initial
88♂♂
111♀♀
CN S3
198♂♂
269♀♀
MI initial
258♂♂
529♀♀
MI S2
%
50
0
50
363♂♂
139♀♀
0-1
2-3
4-5
6-7
8-9
10-11
12-13
14-15
colour value

Fig. 19.

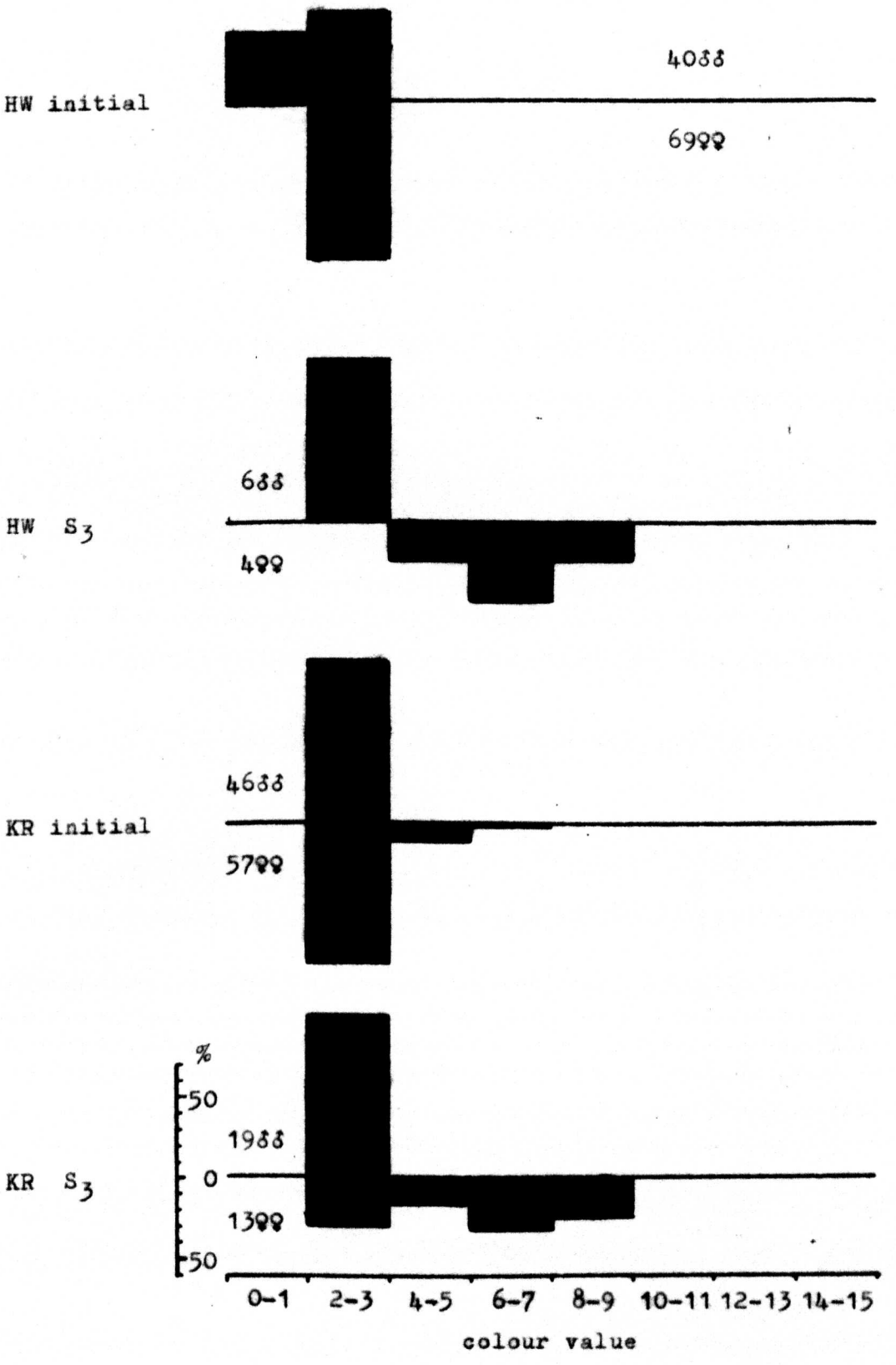
HW initial
4088
6999
HW S3
688
499
KR initial
4688
5799
KR S3
%
50
1988
0
1399
50
0-1
2-3
4-5
6-7
8-9
10-11
12-13
14-15
colour value

Fig. 20.

Figs. No. 21-24

The distribution according to colour value of populations of 16 strains of A. aegypti.

Fig. 21. TV, SA, DC, and TR.

22. BK, SV, TW, and PS.

23. EO, WL, SN, and PN.

24. TN, TA, SO, and NR.

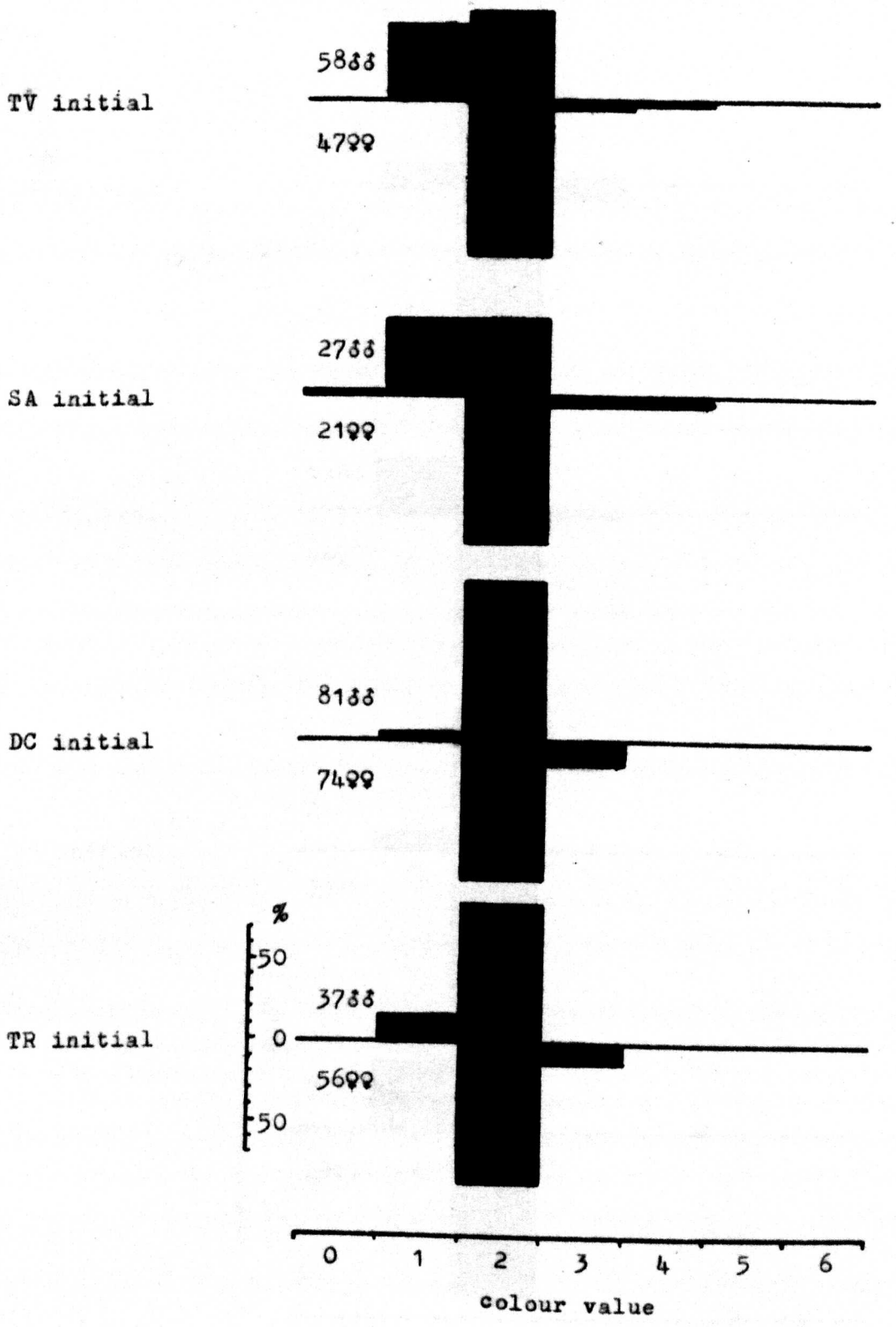
TV initial
5888
4799
SA initial
2788
2199
DC initial
8188
7499
TR initial
%
50
3788
0
5699
50
0
1
2
3
4
5
6
colour value

Fig. 21.

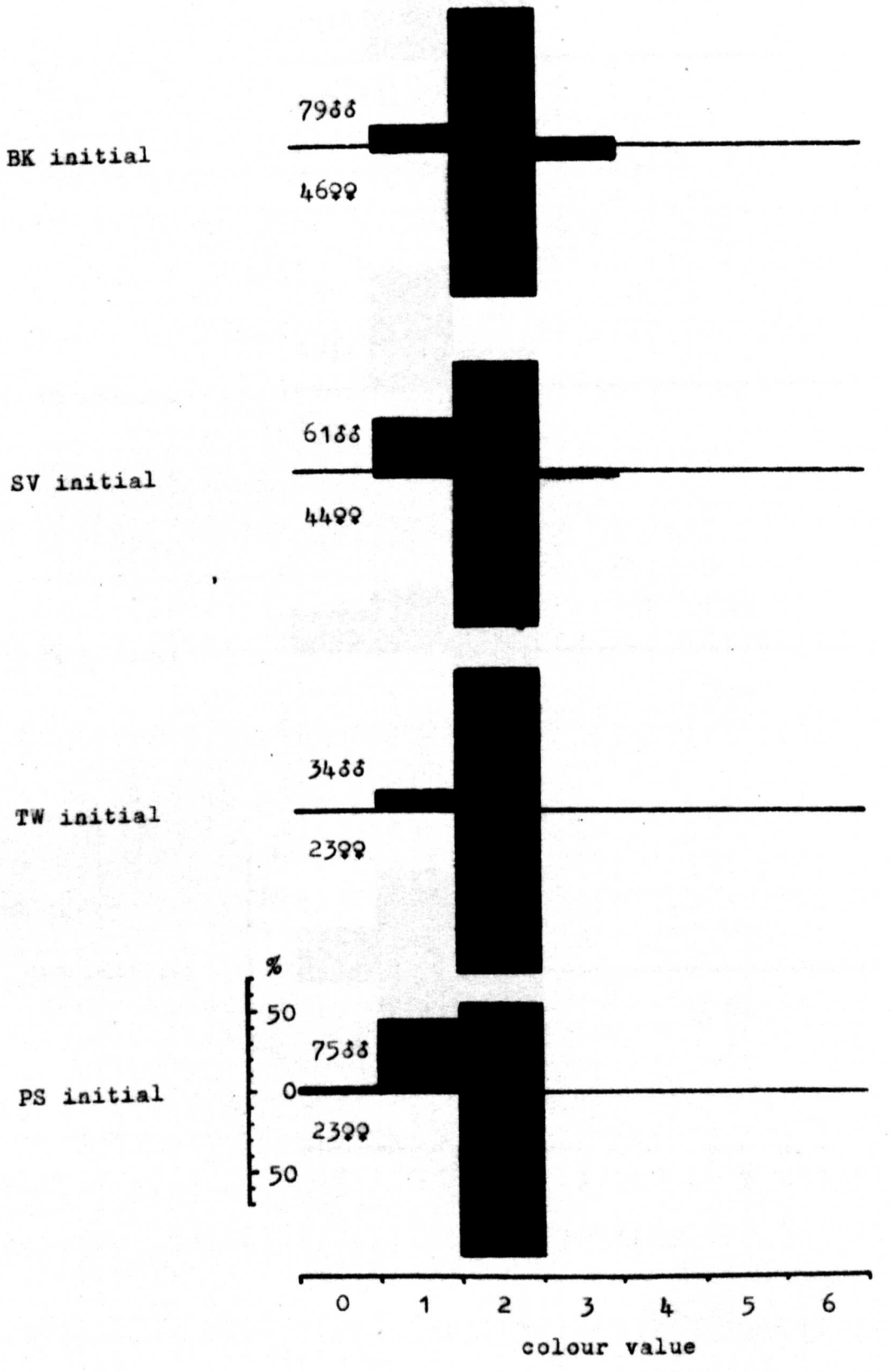
BK initial
7988
4699
SV initial
6188
4499
TW initial
3488
2399
PS initial
%
50
7588
0
2399
50
0
1
2
3
4
5
6
colour value

Fig. 22.

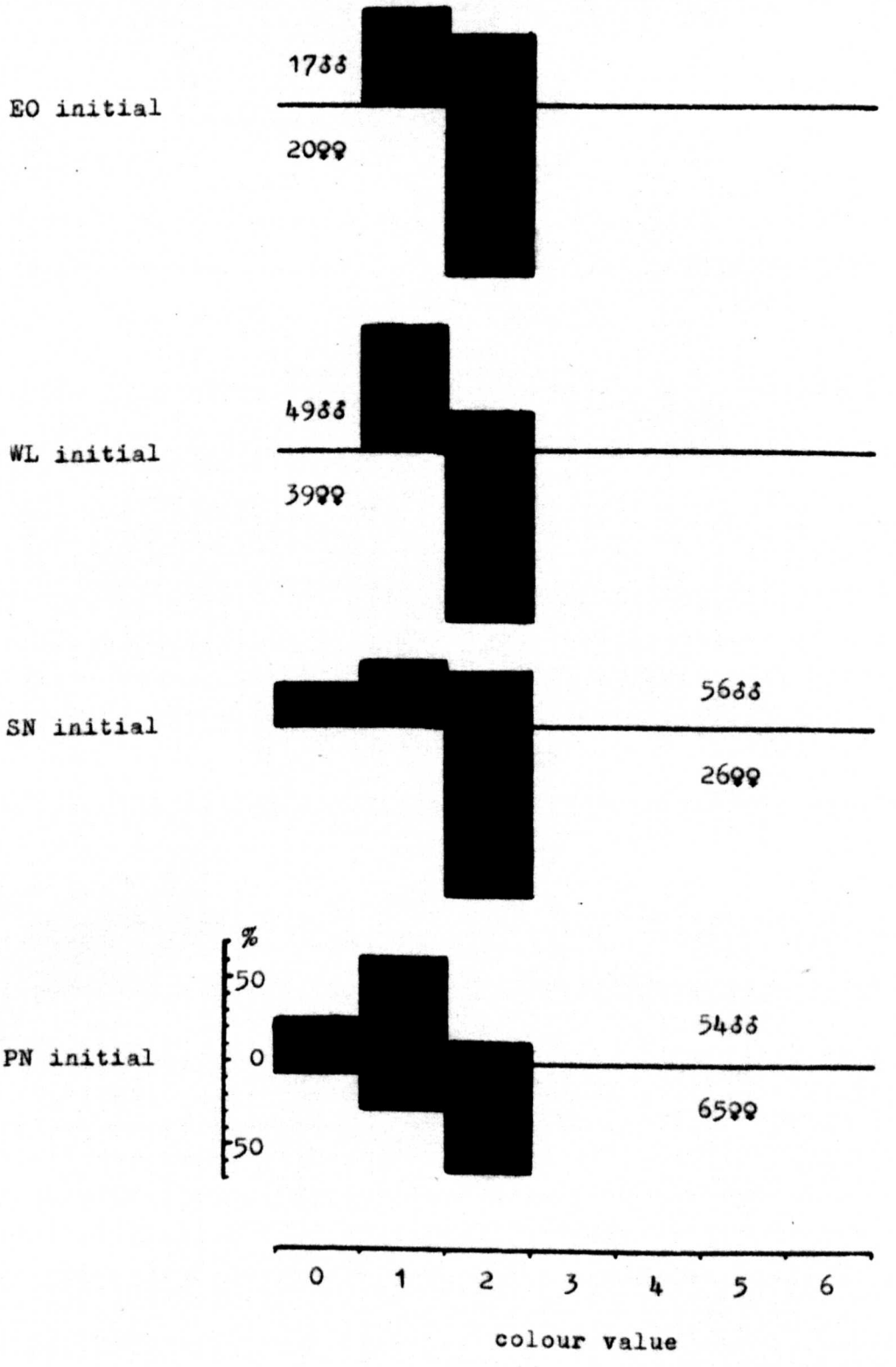
EO initial
1788
2099
WL initial
4988
3999
SN initial
5688
2699
PN initial
%
50
0
50
5488
6599
0
1
2
3
4
5
6
colour value

Fig. 23.

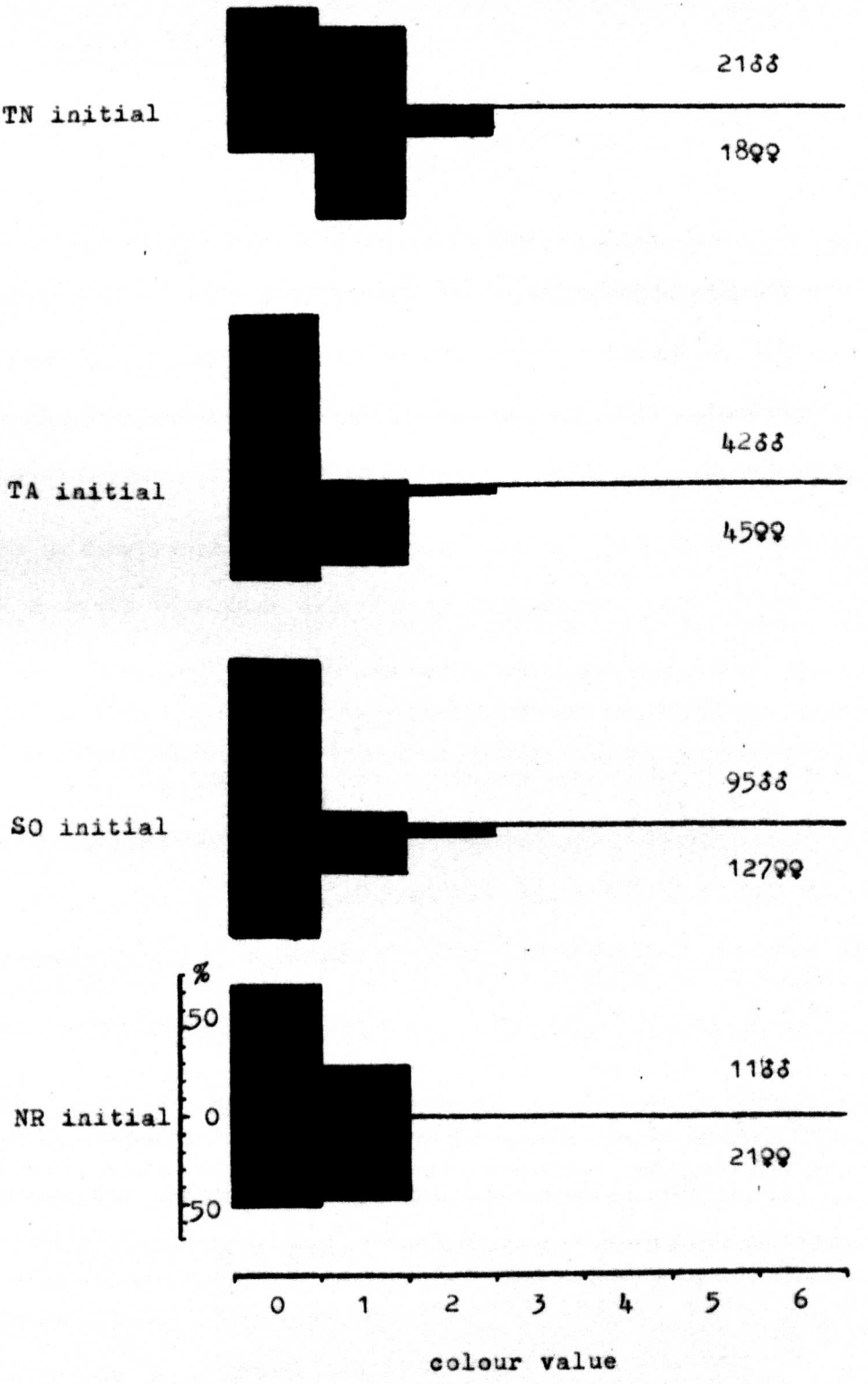
TN initial
2188
1899
TA initial
4288
4599
SO initial
9588
12799
NR initial
%
50
0
50
1188
2199
0
1
2
3
4
5
6
colour value

Fig. 24.

PALENESS CAUSED BY s ALLELES

s^g.

The frequency of the s alleles in populations is of particular interest assuming, as seems most probable, that the similar phenotypes are in fact caused by identical or allelic genes.

The most widespread such "allele" is s^g (or W of Craig) which causes a most obvious degree of paleness even when heterozygous. Ten populations polymorphic for s^g are compared in Figs. 25 and 26. Similar polymorphisms occurred in strains JM, RB, SK and VZ, but numbers examined were too small for fair comparison. The frequency of the s^g homozygotes varied from 0 to 0.8 and is clearly not correlated with laboratory rearing as such. The figures for strain DH might suggest that the genes are in equilibrium in any one colony. The lower frequency of s^g homozygotes in the N.I.M.R. colony, which was subcultured from that in Delhi, might be either the result of random changes if the subculturing resulted in a small population bottleneck, or, equally plausibly, it might indicate a different equilibrium in response to the different conditions of laboratory culture.

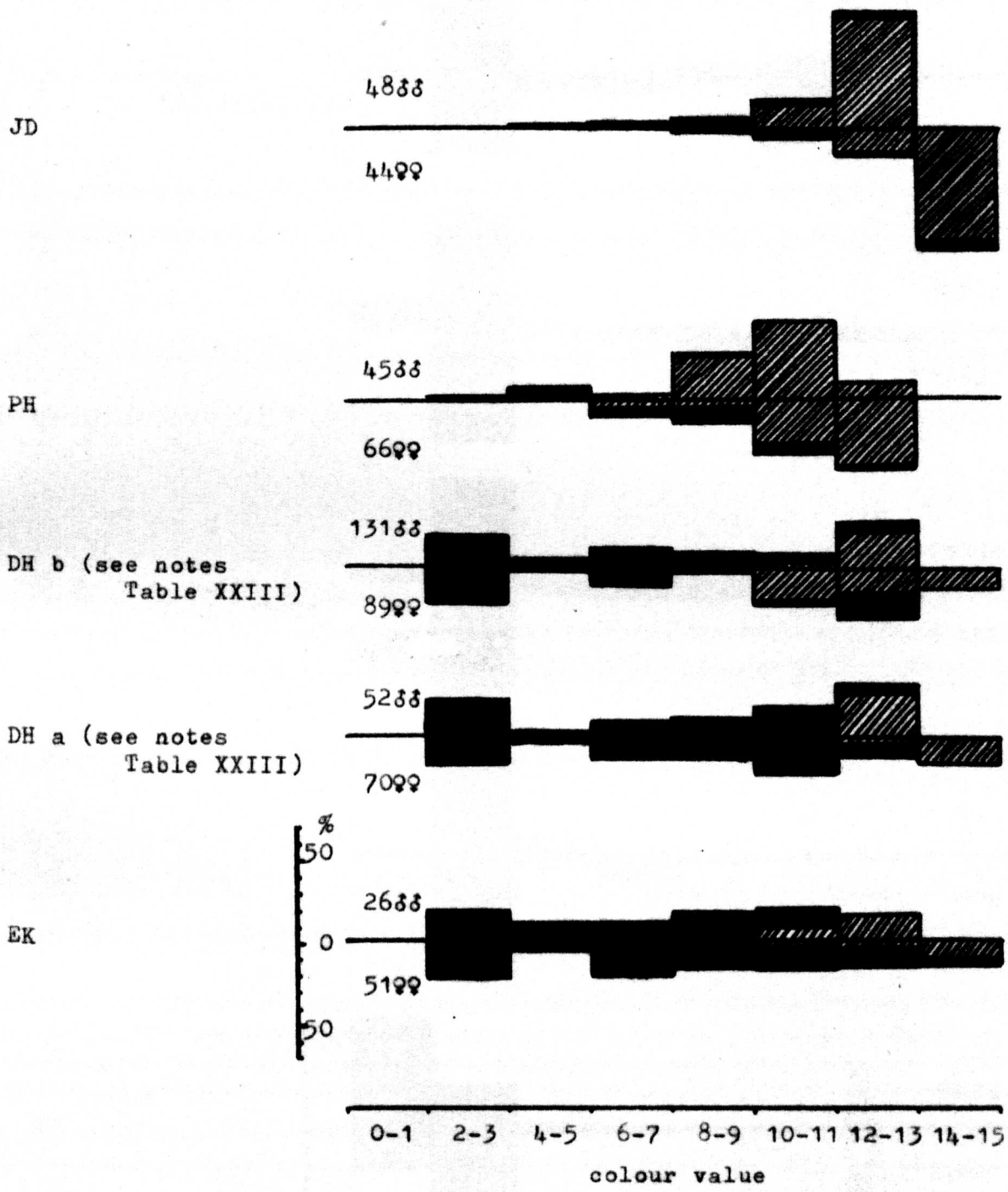

Fig. 25. Distribution according to colour value of populations polymorphic for sg. i) Those with sg frequency of 0.5 or more. black = dominant phenotypes; hatched = recessive phenotypes.

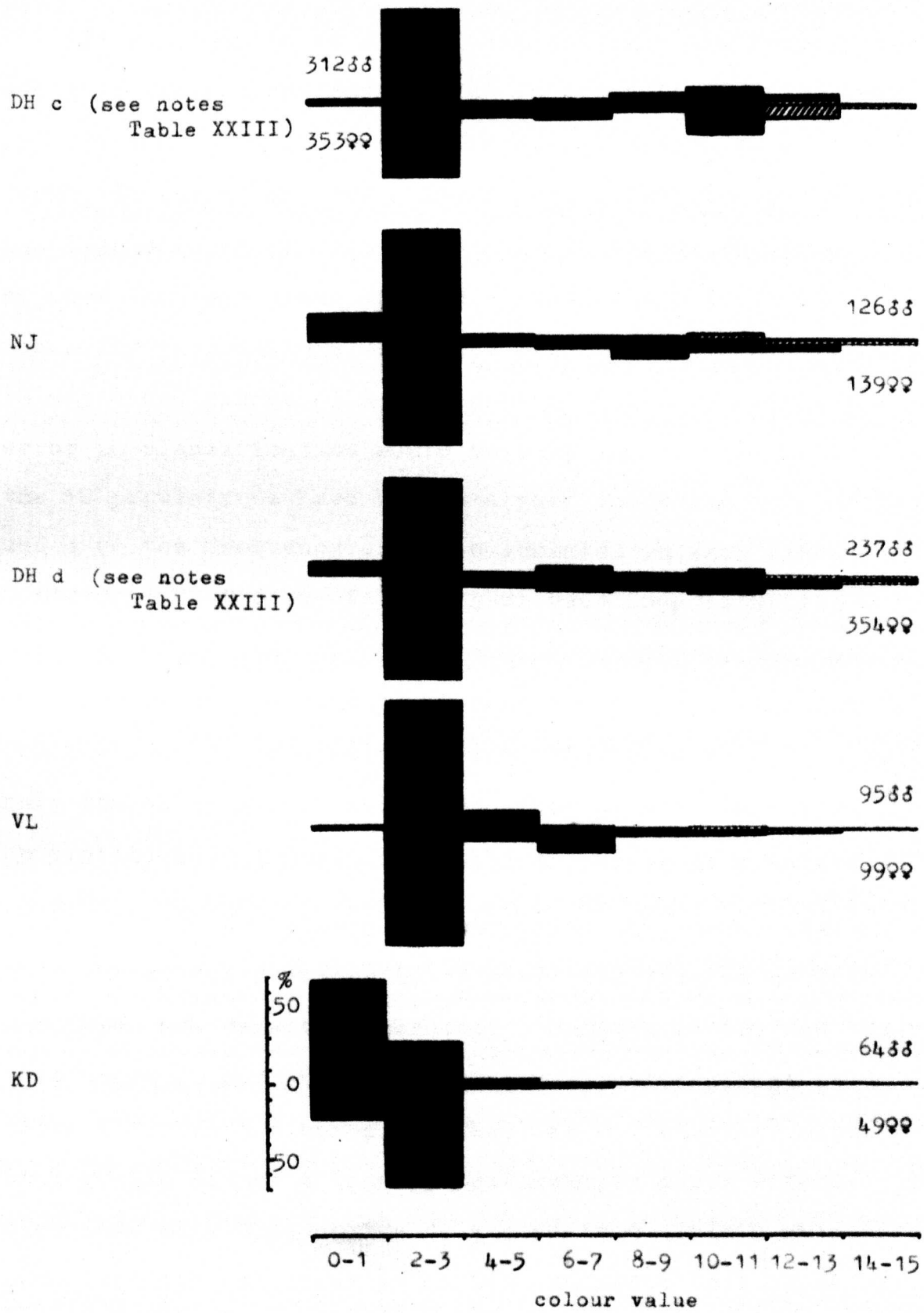

Fig. 26. **Distribution according to colour value of populations polymorphic for s^g.** ii) Those with s^g frequencies less than 0.5. black = dominant phenotypes; hatched = recessive phenotypes.

It is possible to distinguish not only the homozygotes but also the heterozygotes of $\underline{s}^g$ in natural and laboratory populations. In general, $\underline{s}^+$ homozygotes are grade J1 and darker in the males, and grade J2 and darker in the females. The limits in the individual strains do not differ from this by more than one grade and are easily recognized; if necessary test matings were used for confirmation. Since, in any case, grades J1 and J2 contained few examples, the error in classification would be very small. In Table XXIII the 10 populations have been analyzed according to genotype and q (= the frequency of $\underline{s}^g$) calculated in each case. Since some of the populations have been long established in the laboratory (the DH, PH, NJ and KD strains), the continued presence of both $\underline{s}^g$ and $\underline{s}^+$ at none too extreme frequencies indicates a balanced polymorphism, otherwise selection would have tended to eliminate one or other allele (selective neutrality is a theoretical but implausible alternative).

A stable or balanced polymorphism will occur where there is heterozygous superiority or where the fitness of the genotypes are density dependent. HALDANE (1962) has added a case where an autosomal gene has an opposite effect on fitness in the two sexes. There is no reason to suppose that $\underline{s}^g$ has either a density dependent or a sex effect. The fact that high frequencies of $\underline{s}$ seem to correlate with man-made habitats (at least in Africa) suggests that there may be some habitat selection. With habitat selection, fitness of

TABLE XXIII

STRAIN	COMPOSITION OF INITIAL SAMPLE tot no.	$\frac{s^+}{s^+}$	$\frac{s^g}{s^+}$	$\frac{s^g}{s^g}$	GENE FREQ. OF $\frac{s^g}{}$ q	2q(1-q)	NO. OF $\frac{s^g}{s^+}$ EXP.	OBS. MINUS EXP. (dev)	$\frac{dev^2}{EXP.}$ (X^2)	MEAN COLOUR VALUE OF $\frac{s^g}{}$ HOMOZYGOTES ♂♂	♀♀
AO	131	0	0	131	1.00	-	-	-	-	12.9	14.2
JD	102	0	23	79	.89	.200	20.4	+ 2.6	.33	12.0	14.5
PH	112	0	30	82	.87	.232	26.0	+ 4.0	.62	10.0	11.1
DH b	210	48	102	60	.53	.498	105.1	- 3.1	.09	12.1	13.2
DH a	122	22	73	27	.52	.499	60.9	+ 12.1	2.40	12.5	13.9
EK	77	17	45	15	.49	.490	38.5	+ 6.5	1.10	11.7	12.3
DH c	655	363	268	24	.24	.366	239.7	+ 28.3	3.34	12.3	13.2
NJ	267	205	55	7	.13	.225	60.0	- 5.0	.42	10.1	14.1
DH d	591	404	179	8	.17	.276	162.9	+ 16.1	1.59	13.0	13.0
VL	515	414	94	7	.11	.188	96.7	- 2.7	.08	9.7	12.3
KD	113	104	9	0	.04	.076	8.6	+ 0.4	.02	10.8	13.1

TABLE XXIII. Analysis for s^g in different populations.

NOTES DH - a - 1st sample from Indian colony, b - 2nd sample (+1 yr.)

DH - c - 1st sample from N.I.M.R. subcolony,

d - 2nd sample from N.I.M.R. (+ 6 mnths)

$\frac{sg}{s^+}$ expectancies calculated according to the Hardy-Weinberg Rule.

the homozygotes will be greater than that of the heterozygotes. SMITH (1962) has shown that, in the absence of heterozygous advantage, habitat selection could lead to a stable equilibrium; on the other hand, given a stable equilibrium there will be selection in favour of habitat selection.

The expectation of heterozygotes in each sample of N individuals has been calculated in Table XXIII according to the Hardy-Weinberg rule as $2pqN$, where $p = 1-q$. DHc is the only population approaching a significant excess of heterozygotes. Three of the 10 populations fall short of, and 7 exceed, the expected number of heterozygotes. Furthermore, the average χ^2 value for the deviation from expected is 0.20 for the 3 deficient populations and 1.34 for the 7 populations in excess. While these figures fail to satisfy any statistical test of significance, they are nontheless suggestive of some degree of heterosis.

Thus, while $\underline{s}^g$ can be maintained at equilibrium in the laboratory, the selective forces in the field must certainly be very variable to account for its high frequency (0.89) in the Arabian JD strain compared with the low frequency of 0.10 in VL from S.E.India or the intermediate 0.49 in EK from S.W. India. Whether the laboratory populations reflect the gene frequency of the original population is open to doubt.

Other s alleles

The range of variation included under s^w is very large and this may be because more than one allele is involved or because s^w homozygotes are susceptible to modification at other loci as was demonstrated above. Strains KN, MA and MB may be mentioned as particularly interesting examples.

KN originated from egg batches laid by four fertilized females caught biting in a forest at Karen near Nairobi at an altitude of about 1,750 m. Two of the resultant families showed segregation as follows: 16 grade F or G- s^+, 27 grade G to H ap s^+ and 11 grade H-ap to J2 s^w. Both females must have been heterozygous, and fertilized by the same or different heterozygous males. Furthermore, the dark scales of the KN s^w homozygotes had the very intense black that seems typical of inland African subspecies formosus, although the amount of white scaling would have placed them in MATTINGLY's (1957) category of var. queenslandensis.

MA originated as the progeny of 11 single laboratory-mated females reared from wild-caught larvae. s^w segregated in 2 of these 11 families, and 6 out of 7 of the remainder which were selectively bred for a further generation, suggesting that the frequency of s^w in the source population was quite high. Plate IX i shows that not only is the obliqueness of lateral spots strongly emphasized, but there is less tendency for the median pale scaling on the tergites

to aggregate medially as in other strains.

MB originated from larvae found at Miami International Airport and illustrates the enormous potential variation carried within individual mosquitos. The majority of the initial sample lay at or below colour value 2, with grade H- as the mode and 14% in grades F and G-. However, 15% of both sexes were clustered between grade J2 - J4 and the remaining 2% at grades K4 or L3. A J3 female had extended spots like $\underline{s}^{p}$ of strain FS, and the palest male, grade K4, lacked silver lateral spots on the 7th segment again like $\underline{s}^{p}$. This last male was mated with the palest female, of grade L3.

The 41 progeny of this single pair fell into four clearly defined groups, not significantly different from a 1 : 1 : 1 : 1 ratio. The first group comprised 4 H- males and 7 H ap females. The second group, with which the mother could have been classed, comprised 5 males with normal lateral spots varying from grade J3 to L3, and 8 females with normal lateral spots varying from grade J3 to K4. The 5 males of the third group, to which the father had belonged, resembled $\underline{s}^{p}$ and ranged from grades K3 to M2. These were presumably the homologues of a group of 5 females with enlarged lateral spots resembling $\underline{s}^{p}$ ranging between grade L4 and Q. The fourth group comprised 4 typical $\underline{s}^{g}$ males of grade P0 and 3 females of grades N2 and Q. It is clear that at least 2 separate pale-producing factors are involved, but the

segregation could be explained on the basis of 3 alleles at a single locus, for example :-

$$\frac{s^g}{s^+} \times \frac{s^g}{s^?} \longrightarrow \frac{s^+}{s^?} \quad \frac{s^+}{s^g} \quad \frac{s^g}{s^?} \quad \frac{s^g}{s^g}$$

mother father Gp 1 Gp 2 Gp 3 Gp 4

Only two subsequent matings were possible, but these showed that a pair of Group 4 bred true, while a pair of Group 3 segregated into group 3 and group 4 with, in addition, a third group ranging from grade Hap to J4 with the lateral spots also as in $\underline{s}^p$; on the single locus hypothesis this would be $\frac{s^?}{s^?}$. Unfortunately, there was no opportunity to test for allelism with FS $\underline{s}^p$.

DISCUSSION AND CONCLUSIONS

LINKAGE MAP

Table XXIV summarizes the main results of the present investigation and shows that 15 factors have been isolated at 11 loci. Five linkage distances have been determined and a further two estimated. A total of 6 linkage distances have been published previously, but these include separate measurements for both s and W, which are now shown to be allelic.

Particular interest attaches to linkage group I, corresponding to the sex chromosomes. The present work is the first to confirm, outside Culex, the conclusions reached 15 years ago in the same laboratory by GILCHRIST and HALDANE (1947). The sequence and recombination between two genes, ru and re, and the sex factor have been established, the first three-point linkage estimation to be reported in mosquitos. Although Gilchrist and Haldane considered sex in Culex to be determined by a gene or small chromosome section, there seems no reason why the two sex "alleles" should not be homologous with the heterosomes of other Diptera.

The three pairs of chromosomes in the Culicine mosquitos could be derived from the 3 pairs of autosomes and 1 pair of heterosomes of the Tipuloidea, not by the loss of the small heterosomes (WHITE, 1949), but rather by their fusion with a pair of autosomes.

TABLE XXIV

SITE	SYMBOL AND MUTANT NAME	NO. OF LOCI	DOMINANCE WITH RESPECT TO WILD-TYPE	LINKAGE GROUP	PERCENT CROSSOVER
sex	M maleness	1	D to m (?)	I	7.0 from re
head	re red-eye	1	r	I	20.6 from ru
	ru rust-eye	1	r	I	27.6 from M
	ol olive-eye	1?	r	(A)	-
thorax	ds dark scutum	1	r	II	1.9 from s
	Fl Fleck	1?	D	-	-
	St Stripe	1?	D	-	-
abdomen	pa pale abdomen	1	r	I	c.0.5 from M
	s^r, s^p, s^w & s^g; spot	1	r/semi-D	(II)	-
leg	blt^1, blt^2; black-tarsi	1	r	(III)	-
	th tarsi-hooked	1	r	III	21.1 from blt
other	fz fuzzy	1	r	III	<18 from blt

TABLE XXIV. Summary of well-defined mutants and linkage information obtained in the present study.

Notes - r = recessive to wild-type, D = dominant to wild-type. (A) = autosomal. Other parenthesis indicates that linkage group was known for other allele.

While the absence of pairing and disparity of size between separate X and Y chromosomes would be readily observed, it is doubtful if small unequal non-pairing segments of large autosomes (resulting from fusion with the X and Y) would be noticed, especially if they were terminal. If this were so, sex determination in Culicine mosquitos would be in reality of the XX - XY type with a very large homologous pairing section. Most genes on this pair of chromosomes would thus show partial sex-linkage. Proof of this hypothesis would come with the finding of genes showing the normal mother-to-son type of sex-linkage with no recombination between the sexes. Alternatively, if sex is in fact a single gene, it would be surprising if it were always terminal, as inversions could be expected to carry it to the middle part of the chromosome. The finding of partially sex-linked genes on both sides of the sex factor would confirm this. Searches for more sex-linked genes should therefore receive priority. Meanwhile, it is convenient to use the m, M alleles as a working notation.

If the sex-determining entity is terminal, it is unlikely that genes at the other end of the chromosome will show any obvious sex-linkage. Thus the definitions of mutants as autosomal simply on the grounds of absence of sex-linkage must be accepted with caution. For this reason, ru, situated quite far from M, would perhaps be a useful reference point for establishing locations in linkage group I. The fact that y showed no sex-linkage was scant ground,

a posteriori, for placing it in linkage group II (CRAIG and GILLHAM, 1959), but it was nevertheless justified by the later linkage information. Similarly, the finding of *th* at a distance of about 20 from *blt*, and neither obviously linked to *M* or *s*, seems reasonable confirmation that these belong to the third linkage group.

EYE COLOUR

The eye colour mutants are particularly interesting, but subtle changes are difficult to detect. *ol*, for example, had a profound effect in conjunction with *re ru* but was hardly noticeable in its original genetic background. Suspected eye colour mutants should therefore be reciprocally crossed with *re ru* stock and the F_2 searched for unusual recombinants.

It would be interesting to characterize the effects of the eye colour mutants using paper chromatography. It is possible that light may control the subsequent darkening of *re* and *ru*. This could easily be tested by rearing under various levels of illumination. The *re ru ol* genotype is particularly interesting and an attempt should be made to reisolate the colourless-eyed adults to investigate their behaviour and apparent sterility.

The final problem relating to eye pigments raised by this study is the nature of the blue colour occasionally observed. It is possible that such a blue (or yellow-absorbing) pigment is combined with red (or blue-green absorbing) pigments to

produce the apparently black wild-type colour. The various eye pigment mutants so far isolated all give red hues. These may represent some failure to produce the blue component. The blue effect might similarly result from non-production of red pigments. Were genes causing the two effects combined in one genome, the resultant phenotype might be white-eye. Comparative study of the responses of wild-type, red-eyed and perhaps blue-eyed adults to red and blue light might be revealing.

SCALE COLOUR

At least two major loci, s and pa, are involved in controlling the amount of pale scaling on the abdominal dorsum. There is almost certainly more than one pa allele and at least 4 s alleles. The pa alleles are almost completely recessive to wild-type, whereas the s alleles show varying degrees of dominance. Their effect is additive, such that the double heterozygote is paler than either single heterozygote. The abdominal colour of material that is wild-type with respect to both these loci can vary from colour grades F to H. This variance appears to be polygenic and the genes concerned are possibly also responsible for much of the variance in paler s and pa forms.

The contention that the control of abdominal colour is multifactorial (McCLELLAND, 1960b) is therefore fully upheld. CRAIG and VANDEHEY (1962), in discussing the dorsal abdomen of A. aegypti, point out that it is controlled by a single semi-

dominant gene and is therefore not multifactorial, although they admit that s causes "an irregular increase of white scaling". Although the gene concerned, s^g, is certainly semi-dominant and produces, when homozygous, the palest abdominal phenotypes, it would be fallacious to assume that all A. aegypti with white scaling on the abdomen carried s^g. In the present study, genes affecting the amount of white scaling on the abdominal dorsum occurred in more than half the 51 strains studied. CRAIG et al. (1961) examined museum material for G and W phenotypes and concluded that at least one of the genes was present in material from 9 countries. Since they presumably scored heterozygotes, it is obvious that s^g heterozygotes and pa homozygotes would have been confused.

It is gratifying that all the previously described colour varieties of A. aegypti and all the variations noted by CONNAL (1927) were encountered in the present study. The finding of a partially sex-linked factor controlling abdominal colour can explain for the first time the hitherto puzzling claim of SHIDRAWI (1955) that inheritance of scale colour in a certain strain of A. aegypti was maternal.

The colour of the hind tarsi is controlled by at least two alleles at the blt locus, and there is evidence of further variability which could be caused by other alleles or genes at different loci. The clearly monofactorial inheritance of blt^2 contrasts with the results of FLOCH et al. (1942) which are, however, too imprecise for useful comparison.

The genes affecting the mesonotum fall into three groups, (i) recessive, causing various degrees of pattern obliteration, (ii) dominant, causing a paling of the background scale colour and (iii) dominant, causing an increase in the size of the lines of white scales. It is too early to decide how many of these are allelic, but of (i) it can be suspected that ds is allelic with the similar variants later described. In (ii) the degree of paleness of the female mesonotum varies in different strains from straw-colour to golden-brown, and if all are due to a single gene G it must be subject to considerable modification. It seems likely that there are several alleles at G, one of which might perhaps be ds. In (iii) the effect of St and Fl on different pattern-lines is very similar and allelism is a possibility.

Factors affecting the palps, vertex, halteres and abdominal venter have not yet been adequately studied. From the above summary it does seem that relatively few loci are involved in the control of colour patterns, but that each is capable of mutating to many different alleles - the term allele is used here in the classic sense; it would be premature at the present stage of mosquito genetics to venture into a discussion of pseudoallelism, etc. Furthermore, the loci involved in the control of colour of larvae (y), thorax (G and ds) and abdomen (s) seem to be closely linked. CRAIG and VANDEHEY (1962) suggest that var. queenslandensis is characterized by a high frequency of G, W(= s^g) and y.

These are close enough together on the same chromosome to suggest some degree of co-adaptation. Such a grouping would facilitate the polymorphic maintenance of the var. queenslandensis genotype.

The present study has given little answer to the vexed question of the association of pale forms of A. aegypti with man-made habitats. The finding of a second major locus, pa, causing abdominal paleness on a different chromosome, is interesting, since the PR strain concerned was derived from rock hole breeding places in Puerto Rico. It may be that genes at this locus are not associated with domesticity. This might imply that it is not paleness per se that adapts the mosquito for domestic life, but rather a special effect of the s locus. A striking feature of diurnal mosquitos and many other insects in the tropics is the preponderance of forms with bright metallic markings. The subgenus Stegomyia of Aedes is a notable example, Hodgesia, Eretmapodites, are some other such genera in Africa. These contrast with the dull dun-coloured nocturnal genera such as most Anopheles, Mansonia, Aedes subgenus Banksinella, Culex, etc. The environment of human dwellings, particularly of the more primitive type, is characterized by darkness, and here lies the possible connection with the s locus of A. aegypti. s homozygotes lack the metallic silver spots on the abdomen and consequently appear much duller-coloured than the palest pa homozygotes which still possess the shiny lateral spots.

Clearly there is a great need for experiments on the physiology of genetically defined A. aegypti, comparing pale and dark, s^+ and s forms. So far as is known, there has as yet been no such work. The great body of physiological studies on A. aegypti have been conducted on supposedly homogeneous laboratory strains. Responses to radiant heat might be a promising first line of attack. The differential of heat absorption would be much greater between areas of black and highly reflective silver scaling than between the black and dull-white scaling characteristic of s^g homozygotes. The possibility of associated sense-organs could also be investigated.

Like most genetic studies, the present tends to make nonsense of attempts at clear-cut definitions of the species concerned. The type form of A. aegypti was defined by MATTINGLY (1957) with full regard for the variation in the species, unlike most type forms which describe some arbitrarily chosen single specimen. Mattingly's definition does therefore come near to the idealized wild-type which could be defined as possessing at every locus that allele with the highest frequency in the species. Certainly more A. aegypti resemble the type form than differ from it noticeably. Mattingly is probably right in considering the darker form as a subspecies, since it is so characteristic of the greater part of inland sub-Saharan Africa. The occurrence of some forms as dark as ssp. formosus in the Caribbean, Indonesia, etc.

and some paler forms in the heart of Africa need not lessen its value as a subspecies, providing the non-African forms show the peri-domestic habit and the paler African forms retain feral behaviour-patterns. On the other hand, as has been suggested above, the definition of MATTINGLY (1957) was a little too stringent.

The third form, var. queenslandensis, poses a different problem. Although it is usually taken to be a very pale form, Mattingly's definition is so wide as to be almost meaningless, including as it does all individuals paler than the type form. Genetically, the pale colour of these could result from a variety of genotypes. Furthermore, the total variance within the definition of ssp. formosus and the type form lies between colour grades H ap and F (see Plate I), while all the rest is var. queenslandensis. This makes a very disproportionate grouping with the other two forms. The writer would like to plead that the scheme of colour grades and values suggested above be adopted by workers using or describing A. aegypti and requiring to record that most variable character, colour of the abdomen. Colour of individuals can be specified with great precision by grade and that of groups by mean colour value, while attempts at taxonomic definition are avoided. SENEVET and ANDARELLI (1961) have already classified A. aegypti from various regions using the earlier simplified scheme (McCLELLAND, 1960b).

PART IV

OTHER STUDIES AND FINAL REMARKS

OTHER STUDIES

HYBRIDIZATION WITHIN THE SUBGENUS STEGOMYIA

Eggs of 7 species of the sub-genus Stegomyia besides A. aegypti were received from the Ethiopian region. As a rule, an attempt was first made to maintain the species in the laboratory by force-mating before investigating the possibility of hybridization. If this failed, hybridization would probably have failed also, whereas if all the often small initial batches were depleted in unsuccessful attempts at cross-mating to one other species there would be no means of repetition with another species. The interspecific crosses are summarized in Table XXV and detailed below. Only three crosses produced hybrids and only two, fertile hybrids. The significance of the A. simpsoni x A. woodi cross is beyond the province of the present thesis, but the case of A. aegypti x A. mascarensis, with its bearing on genes controlling colour pattern, merits more extensive discussion.

CROSSES IN BOTH DIRECTIONS YIELDING FERTILE HYBRIDS

1. A. AEGYPTI x A. MASCARENSIS

Introductory

A. mascarensis MacGregor (Plate XV j) is one of the most

TABLE XXV

MALE SPECIES	FEMALE SPECIES							
	A. aegypti	*A. simpsoni*	*A. mascarensis*	*A. woodi*	*A. metallicus*	*A. apicoargenteus*	*A. deboeri*	*A. albopictus*
A. aegypti	F ~	+ ^	F ^	-	-	0	0	0
A. simpsoni	-	F ^	-	F ^	-	-	0	-
A. mascarensis	F ^	-	F ~	0	0	0	0	0
A. woodi	-	F ^	0	F ^	0	0	0	0
A. metallicus	0	-	0	0	F ^	0	0	0
A. apicoargenteus	0	-	0	0	0	F ^	0	0
A. deboeri	0	-	0	0	0	0	F ^	0
A. albopictus	-	-	0	0	0	0	0	F ~

TABLE XXV. Summary of interspecific matings in *Stegomyia*

NOTES - F offspring of both sexes fertile
+ offspring obtained, but sterile
- no offspring obtained
0 cross not attempted
~ free mating in cage
^ force-mating by hand

interesting species of the sub-genus Stegomyia known only from the island of Mauritius in the Indian Ocean, where it was first discussed and described by MACGREGOR (1923). He emphasized the whiteness of the scutum, although his actual description of the ornamentation omits reference to colour. Other striking features overlooked by MACGREGOR (1923, 1927) in both his descriptions were the lack of pale knee-spots on all femurs and the absence of pale scales on the posterior pronotum and paratergites (EDWARDS, 1941), which is unique within the sub-genus, although MATTINGLY (1953) found a few pale scales on the posterior pronotum. Edwards' description also added the absence of pale scales on the female clypeus which is shared by all Stegomyia except A. aegypti and A. vittatus (MATTINGLY, 1957). The precise extent of the scutal pale scaling was left in doubt by the early descriptions and in specimens available to MATTINGLY (1953) the area was rubbed. All agreed, however, that the typical anterolateral spots are visible as patches of more densely white and broader scales than the background. MATTINGLY (1953) further points out that, although the median anterior spot and the median longitudinal pale lines are indistinguishable, the postero-lateral lines and supra-alar patches are visible.

In its larva (MACGREGOR, 1927) and the male terminalia (EDWARDS, 1941) A. mascarensis is virtually indistinguishable from A. aegypti. In spite of this, Edwards, having originally placed it in a group of its own among African

Stegomyia (EDWARDS, 1924) suggested its affinity with the oriental species such as Aedes annandalei and A. w-alba which are rather similarly pale scaled.

It was left to MATTINGLY (1953) to excite further speculation on this species and recognize its close affinity with A. aegypti in group A, together with a new species he described as A. vinsoni, from a single female discovered in Mauritius by J. Vinson in 1946. MATTINGLY (1953), noting in A. vinsoni the similarities of the thorax to A. mascarensis and of the abdomen to the pale var. queenslandensis A. aegypti, suggested that the possibility of hybridization between the two be investigated.

MATTINGLY and BRUCE-CHWATT (1954) term A. vinsoni and A. mascarensis "the two closest relatives of A. aegypti to be found anywhere in the world". MATTINGLY (1956) suggested that both might be recently derived island forms of A. aegypti, quoting Halcrow's opinion that the latter may have been introduced to Mauritius by the Arabs in about 1000 A.D.; A. vinsoni might "represent the type of aberrant form which tends to appear towards the end of an eradication campaign". MATTINGLY (1957) later considers A. mascarensis as just the sort of form he would postulate as a Southern Palearctic (as against an Ethiopian) ancestor of A. aegypti. All the more obvious points of difference between A. mascarensis and A. aegypti are summarized in Table XXVI.

TABLE XXVI

CHARACTER	DESCRIPTION	
	A. mascarensis	A. aegypti
Clypeus	bare	white-scaled
Median stripe on vertex	broad	narrow, or obscured by much pale scaling
Anterior median spot	absent or obscured	present
Anterolateral spots	semi-lunar	narrowly crescent
Background scaling of anterior scutum	white	black
Median paired lines	absent or obscured	present
Posterolateral lines	abbreviated	extend to scutellum
Lines either side of and in front of the prescutellar bare space	absent	present
Paratergites	bare	white-scaled
Pleural scale pattern	white spots reduced	white spots large
Femoral "knee-spots"	absent	present
Abdominal dorsum	1st tergite all black as in A. aegypti ssp. formosus	1st tergite usually white-scaled, except in ssp. formosus
Behaviour	sylvan, tree-hole breeder, as A. aegypti ssp. formosus	largely associated with manmade habitats, except ssp. formosus

TABLE XXVI. Points of difference between A. mascarensis and A. aegypti.

Results

strain maintenance

Initial attempts to maintain this species by hand-mating failed after 3 generations, but not before its successful use in hybridization studies. A second consignment of A. mascarensis, received in 1962, has been more successfully maintained as a mass colony by a modified technique involving a larger "Perspex" cage, a lower rearing temperature of 24°C and black-paper oviposition sites. As this material was not used for hybridization it will be discussed separately in a later section.

variation in the 1961 material

Some of the adults reared from the first eggs received from Mauritius showed a reduction in the amount of white ground scaling of the anterior scutum as in the male illustrated in Plate XV n. Of 49 adults reared 11 males and 13 females were thus dark-scaled, the remainder conforming to the type description. A single pair dark x dark mating gave a progeny of 12 dark and no pale males, 17 dark and 12 pale females. A single pair pale x pale mating gave 17 dark and 9 pale male offspring and 11 dark and 9 pale females.

F_1 hybrids with A. aegypti

Because of the effort to culture the species, only 6 males and 1 female could be spared for hybridization attempts

using a total of 11 females and 1 male of A. aegypti. Fertile eggs were obtained from the single A. mascarensis female and 5 of the A. aegypti. The hybrids were as follows:

a) A. aegypti female, strain GA re x pale A. mascarensis male. One pair giving 37 male and 23 female hybrids. These differed from typical A. aegypti only in the slightly broader anterolateral spots, the slightly shorter white lines bordering the prescutellar bare space, and, most noticeably, the speckling of white scales on the anterior part of the mesonotum (Plate XV m).

b) A. aegypti female, strain GA re x dark A. mascarensis male. Two pairs, one giving 15 male and 22 female hybrids, the other a larger number. All the hybrids examined resembled those from (a) without the speckling of white scales and were thus virtually indistinguishable from A. aegypti.

c) Pale A. mascarensis female x A. aegypti male of strain JD s^g (also with the gene G, Gold mesonotum). One pair giving a large hybrid progeny differing from (a) in the abdomen which resembled the usual s^g x wild-type heterozygote in A. aegypti, and in the mesonotum of the females in which the white scales speckled a brown, as against a black, ground.

d) A. aegypti females of strain JD s^g, as in (c), with a gold mesonotum x dark mascarensis males. Two large hybrid progenies obtained which differed from those of (c) only in the absence of the white speckling.

Although no reciprocal crosses using exactly the same classes of parent were made, comparing the (c) hybrids with the others, there is no indication of any non-reciprocal effects.

subsequent hybrid generations and F_2 analysis

Backcrossing the aegypti-mascarensis hybrids to the "dominant parent", A. aegypti would have given no segregation and the A. mascarensis had been lost, so the F_2 generation of (a), (b) and (c) hybrids were analysed. These had mated freely in the standard Barraud cage, but some of the (b) hybrids were force-mated as a safeguard.

Using tentative gene symbols, and classing the mascarensis characters as recessive or semi-dominant mutants, the F_2 adults were scored for the most obvious three. Tw or Tw^+, white scaling on the anterior mesonotum, more than, as against not more than that in the F_1 hybrids. sl or sl^+, anterolateral spots approaching a semi-lunar shape as against not broader than those of the F_1 and ks or ks^+, white "knee" spots absent or present. The result of this analysis is given in Table XXVII. Additional segregants appearing in the F_2, often in combination with one or other "mascarensis"

TABLE XXVII

PHENOTYPES	F_2 offspring from *A. mascarensis* x *A. aegypti*					
	from (a) hybrids		from (b) hybrids		from (c) hybrids	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
Tw sl ks	2	1	1	-	-	-
Tw sl +	-	1	1	-	-	-
Tw + ks	3	4	1	1	-	-
+ sl ks	1	-	5	-	-	-
Tw + +	2	2	8	19	-	-
+ sl +	6	-	21	3	3	-
+ + ks	5	15	10	6	4	1
+ + +	47	35	65	86	11	21
TOTALS	66	58	112	115	18	22

TABLE XXVII. Three character analysis of F_2 hybrids between *A. mascarensis* and *A. aegypti*

character, included $\underline{s}^{w}$, $\underline{s}^{g}$, re, blt and th which had all been present in one or more of the A. aegypti parents.

With two exceptions, the frequency of the individual characters is considerably less than the 25 per cent expected on the assumption of monofactorial inheritance. Multifactorial inheritance is possible in the case of Tw in the (a) and (c) hybrids, since only a small proportion were as pale as the parent A. mascarensis; as emphasized above, all scored as Tw were paler than F_1 hybrids. The number of sl is mainly deficient in the females, probably because the difficulty of drawing a distinction between broad F_1-type crescents and narrow semi-lunar spots is greater in females than males, and doubtful cases were counted as F_1 types. sl might also be multifactorial or possibly controlled by a single partially dominant gene of variable expression. The total frequency of ks is only 10.6% in the F_2 from cross (a) due to non-scoring of all cases where even single pale scales were present at the tips of the femurs. In the F_2 from cross (c) the absence of an obvious (aegypti-like) knee spot was scored as ks and the total frequency is exactly 25%. Moreover, there is a sex bias in both cases giving more ks in the F_2 progeny of the same sex as the A. mascarensis grandparent. The bias is significant below the 5% level of P in both cases (cross (a), $\chi^2_c = 4.04$; cross (c), $\chi^2_c = 4.32$ n = 1) and probably indicates that ks is sex-linked.

Following the loss of the A. mascarensis material, a totally unsuccessful attempt was made to resynthesize the "species" from F_2 segregants. Selective matings for Tw invariably failed. Although examples of the F_1-type white speckling persisted to the F_6 from cross (c), no segregating s^g homozygotes ever showed it. Some individuals were homozygous for both ks and blt, but an attempt to select such a line failed. It is certain that none of the F_2 segregated for naked paratergites (designated pt) but in the F_4 from cross (b), after selection for ks, one family gave 10 pt males and 14 pt females out of a total of 31 males and 25 females (pt and pt^+ examples from this family are compared in Plate XV p and q.). Unfortunately the F_3 was not carefully scrutinized for this character.

Two of these selected lines, now at about the F_{10}, have been expanded and stabilized as colonies, one homozygous for ks with some segregation of pt but otherwise like A. aegypti. The other was selected for sl, and all individuals resemble A. aegypti except for the broader anterolateral spots. Examples from this line, showing the full semi-lunar expression illustrated in Plate XV o, are however rare; the prominent development of the posterolateral lines should be noted.

variation in the 1962 material

The first two of three consignments of A. mascarensis, received direct from Mauritius, were pooled and the adults

simply sorted as Tw or Tw^+ following the previous year's practice. Each of these two groups mated freely in a Barraud cage to produce a "Tw" or a "Tw^+" F_1.

The adults from a third consignment were then examined more closely. Many of these departed in several ways from the type description of A. mascarensis but always in the direction of A. aegypti. The adults were scored as Tw or Tw^+, sl or sl^+, ks or ks^+ and pt or pt^+ as before, and also as cy or cy^+ depending whether pale scales were absent or present on the clypeus. Thus type A. mascarensis would be scored as Tw - sl - pt - ks - cy, and A. aegypti as Tw^+ sl^+ pt^+ ks^+ cy^+. This search revealed such remarkable variance that the "Tw F_1" and "Tw^+ F_1" were similarly analyzed and the results are compared in Table XXVIII. Combination of the "Tw" and "Tw^+" F_1 data probably removes much of the bias due to the original selection, and as the origin of all three lots was the same small forest area in Mauritius (R. MAMET, personal communication) summation of the data of Table XXVIII is probably valid.

Of the total of 272 individuals only 21.4% were type A. mascarensis, 42.7% varied in one of the 5 characters, 23.9% in 2, 11.5% in 3 and 0.8% (two females) varied in 4 of the characters, and would have passed any scrutiny as A. aegypti. 97.1% were homozygous for cy, 87.5% for ks, 87.1% for pt, 66.9% for sl and 33.8% homozygous for Tw. A female scored as Tw^+ sl^+ pt^+ ks cy is illustrated in Plate XV 1,r.

TABLE XXVIII

PHENOTYPES	FIELD BATCH		"Tw F_1"		"Tw+ F_1"		TOTAL	PERCENT OF GRAND TOT.
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀		
Tw sl pt ks cy	-	4	12	24	11	7	58	21.4
+ sl pt ks cy	4	2	18	6	43	21	94	34.6
Tw + pt ks cy	2	5	1	4	-	-	12	4.4
Tw sl + ks cy	-	-	-	1	-	-	1	0.4
Tw sl pt + cy	1	-	3	3	-	-	7	2.6
Tw sl pt ks +	-	-	-	2	-	-	2	0.7
+ + pt ks cy	5	12	8	8	-	6	39	14.4
+ sl + ks cy	5	1	1	-	2	-	9	3.3
Tw + + ks cy	2	-	-	1	-	-	3	1.1
+ sl pt + cy	1	2	2	-	-	-	5	1.8
Tw + pt + cy	4	-	-	1	-	-	5	1.8
Tw sl + + cy	-	-	1	-	-	-	1	0.4
+ sl pt ks +	1	-	-	-	-	-	1	0.4
Tw + pt ks +	-	1	-	1	-	-	2	0.7
+ + + ks cy	7	6	1	-	-	1	15	5.5
+ + pt + cy	3	4	1	2	-	-	10	3.7
+ sl + + cy	2	-	1	1	-	-	4	1.5
Tw + + + cy	1	-	-	-	-	-	1	0.4
+ + pt ks +	-	-	-	-	-	1	1	0.4
+ + + ks +	-	-	-	-	-	1	1	0.4
+ + pt + +	-	1	-	-	-	-	1	0.4
TOTALS	38	38	49	54	56	37	272	

TABLE XXVIII. Genetic analysis of A. mascarensis for 5 characters

Of incidental interest in this material was a male showing unilateral absence of the whole antenna (Plates XV n,XVI d) with the site of the torus invaded by extra ommatidia.

The relation of A. mascarensis to A. aegypti

The genetic compatibility between the two species immediately raises the question of the specific status of A. mascarensis. Could it be simply a variety of A. aegypti ? The results of the F_2 segregation of the aegypti-mascarensis hybrids, and the separate combinations of various characters in the 1962 material indicates that the differences between the two species are controlled at several independently assorting loci. If other characters such as the vertex stripe, pale scales on the pleurae, posterolateral lines and those around the prescutellar bare space are considered, the number of loci necessarily involved increases. To this may be added differences in habit, A. mascarensis is a truly sylvan mosquito preferring the higher parts of the island (MACGREGOR, 1927). It also has the ability to compete successfully with A. albopictus, apparently not shared by A. aegypti, in Malaya at least (MACDONALD, 1956; MATTINGLY, 1957), because in Mauritius larvae of A. mascarensis and A. albopictus normally share the same tree-hole breeding place (MACGREGOR, 1923, 1927). The possibility that A. mascarensis is merely a polymorph of A. aegypti may therefore be discounted. Furthermore the larvae of A. mascarensis were never found in the same breeding place as

A. aegypti, which was, at least in MacGregor's time, restricted to the coastal and lower parts of the island (MACGREGOR, 1923, 1927). The isolation of the two species was not however as sharp as MATTINGLY (1953) postulated since there are A. mascarensis labelled "In houses, Port Louis, J. G. Halcrow 30-1-53" in the British Museum. HALCROW (1954) reported A. aegypti at 1,200 ft. where it last occurred before its final eradication.

Since then more than ten years of surveillance have failed to reveal the presence of A. aegypti or any change in habits or status of A. mascarensis (R. MAMET, personal communication). It is hard to believe that MacGregor, in his years on Mauritius, could have overlooked variation in A. mascarensis as now reported; on the other hand, the confusion of such forms with A. aegypti would not accord with the clear distinction of breeding places. That such variation actually occurs in adult A. mascarensis in Mauritius and is not an artifact of laboratory rearing in London (an unlikely hypothesis) is confirmed from examination of 71 specimens collected in 1961 by R. Mamet. It therefore seems likely that the incidence of these variants is increasing, even the 1962 lot of living material analysed above varied more than that obtained the previous year.

It is hard to escape the conclusion that the "aegypti-like" characters in the population of A. mascarensis are controlled by homologous or identical genes for those of

A. aegypti, but harder still to explain their presence. It is not plausible that so many dominant mutations, each coinciding with a character possessed by A. aegypti and none other, should have arisen independently in a closely related species. Alternatively, these genes might be relicts in a case of incipient speciation. The recently eradicated population of A. aegypti in Mauritius was probably one or both of the paler urban forms (MATTINGLY, 1953, 1957) which spread round the world with man but nowhere, outside the African mainland, evolved truly feral, let alone sylvan populations. The possibility that not only did such speciation happen in Mauritius in less than the thousand years suggested since the probable introduction of the urban A. aegypti (vide supra), but that in the presence of A. albopictus there arose a form as distinct from A. aegypti as A. mascarensis, would be remarkably rapid evolution. If it had, it is even more surprising that the eradication of the initial form should seem to lead to a reversal of the process. A third possibility may now be suggested.

In the colour of its abdomen A. mascarensis resembles not the type form or var. queenslandensis but the feral ssp. formosus of A. aegypti (MATTINGLY, 1957) and other closely related species on the African mainland. It seems possible that A. mascarensis developed in isolation from an ancient population of A. aegypti or some common ancestral form. The reverse, that A. aegypti and perhaps the other Stegomyia

in Africa might have arisen from something like A. mascarensis has been referred to above. In either case, later reintroductions of man-adapted urban forms of A. aegypti into Mauritius would have been virtually isolated from sylvan A. mascarensis until recent urban expansion, and perhaps the effect of control measures may have stimulated some overlap in their distribution. Under these conditions hybridization and consequent gene exchange is a distinct possibility.

If some of the "recent" aegypti genes have a higher selective value in the changing environment of Mauritius, their frequency will rise. The fact that the mascarensis background confers the ability to survive with A. albopictus in the sylvan environment, together with the continued use of insecticides in urban areas, may explain that the rise in frequency of the "aegypti" genes is unaccompanied by any reports of a recrudescence of an urban Stegomyia. If the interpretation is correct, the aegypti-mascarensis population should nevertheless contain a potential for urban habit that A. mascarensis never possessed.

The failure of hybrids between the pale form of A.aegypti and A. mascarensis to produce any combination of thoracic and abdominal paleness clearly discounts the possibility of such a hybrid origin for A. vinsoni. Critical examination of the type specimen shows much clearer affinities with A. albopictus. The recent collection of a mosquito intermediate between A. vinsoni and A. albopictus confirms this (MATTINGLY, in press), and the finding of a similar albinoid A. simpsoni in

East Africa (MATTINGLY, in press) raises the whole fascinating question of the significance of pale forms in A. aegypti/ mascarensis, A. albopictus, A. simpsoni and other species.

2. A. SIMPSONI x A. WOODI

The ease with which Aedes simpsoni (Theo.) (Plate XIV e) can be maintained by hand-mating has led to its use for much attempted hybridization. Adults feed readily and oviposit well, although attempts to get a second egg batch by feeding again have nearly always failed. Three separate lots of eggs were received from Kenya, at different times and from different localities. These were combined in a single "population" to avoid the effects of inbreeding. Not less than 10 egg batches from different pair-matings are hatched together for each generation. Pupae from these are picked at random and no fewer than 20 adult females inseminated.

A. simpsoni is widely distributed and often very common in sub-Saharan Africa; it is a proven vector of yellow fever (MAHAFFY et al., 1942) and its proclivity for breeding in plant axils which are particularly abundant in plantations and hedges, brings it into close contact with man.

Aedes woodi Edw. (Plate XIV g), on the other hand, is apparently confined to a part of Nyasaland and a few small areas near the coast of Kenya and Tanganyika where it is nowhere abundant. This is probably because it seems restricted to breeding in the axils of a single species of swamp sedge Cyperus grandis (HARPER, 1955). Nevertheless, the

genitalia of A. woodi and A. simpsoni seem indistinguishable and the adults and larvae are separable by few characters (MATTINGLY, 1953).

Several egg batches of A. woodi were received from Kenya. The larvae did not thrive well in the laboratory, but produced enough adults for force-mating. Several F_1 pairs were similarly force-mated, but all eggs laid by F_2 females failed to hatch.

6 females of A. simpsoni were easily force-mated with males of A. woodi, 2 of the resulting 5 egg batches being fully fertile. The only female of A. woodi available for the reciprocal cross laid a fully fertile egg batch. The hybrids were nearer to A. simpsoni in pattern than to A. woodi (Plate XIV h).

The woodi/simpsoni hybrids of both sexes were backcrossed to A. woodi, 5 such backcrosses gave a total of 38 progeny, indicating lowered fecundity. The reciprocal simpsoni/woodi hybrids were paired together giving an F_2 of 73 males: 59 females. In both the backcross and F_2 families characters of A. woodi and A. simpsoni assorted independently, thus the narrow anterolateral spots of A. woodi were combined with the pale-scaled lateral scutellar lobes of the hybrid or A. simpsoni (Plate XIV i) or the recessive dark-scaled lateral scutellar lobes of A. woodi were combined with the broadly "pear-shaped" anterolateral spots of the hybrid or A. simpsoni. An effort to breed a "synthetic" form from either of these

recombinants failed.

CROSS YIELDING STERILE HYBRIDS IN ONE DIRECTION ONLY

A. SIMPSONI x A. AEGYPTI

Males of A. simpsoni effectively inseminated 59 female A. aegypti, less than 20 of which laid eggs, mostly small batches and all infertile. This contrasted with the reciprocal cross in which 94 out of 126 female A. simpsoni apparently inseminated by male A. aegypti, oviposited mostly normal-sized egg batches, of which 10 contained at least 2 fertile eggs. The total of eggs hatching was not less than 33 (some mortality of freshly-hatched larvae might have been overlooked) and development proceeded normally with only 5 larval, and 2 pupal, deaths. The pupal sex-ratio was 13♀♀ : 15 ♂♂. All the 13 adults of each sex were identical with respect to thoracic markings which were intermediate between the two species (Plate XIV f). Eight different strains of A. aegypti were used; VL, MA, KN, PR, AO, NJ, SK and GA x BLTS; only the first 3 gave fertile hybrids. In the case of VL and MA, s^g or s^w were mostly used and all of the resulting 15 progeny showed the typical heterozygous expression of s^g or s^w on the abdominal tergites. The banding on the metatarsi of A. simpsoni (Plate XII f) differs from that of A. aegypti (Plate XII b) in the absence of any pale scales on the 4th segment and increased pale banding on the 3rd. The hybrid between A. simpsoni and A. aegypti with wild-type tarsi

(strain VL s^g) shows (Plate XII g) an intermediate condition, with a pale band on the basal third of the 4th segment compared with the basal half in A. aegypti wild-type. In a hybrid with A. aegypti of strain KN homozygous for blt^2 (var. luciensis) (Plate XII c), the same pale band is reduced to the basal quarter (Plate XII h). 37 attempts to mate A. simpsoni females with blt^1 homozygotes of A. aegypti (var. atritarsus) of the SK and GA x BLTS strains failed. The only abnormality noted in any of the hybrids was a shortened labium in one female (Plate XVI e), the labellae and stylets were apparently normal. The few attempts to obtain an F_2 hybrid generation by force-mating, and to similarly backcross the hybrids to either parent, were all negative suggesting sterility, but the number of trials does not permit a significant conclusion.

OTHER NEGATIVE RESULTS

1. A. AEGYPTI x A. ALBOPICTUS

The conflicting results of earlier workers have already been reviewed. The strain of A. albopictus (Skuse) (Plate XIV a) used came from Madagascar - its nearest approach to Africa. It has bred vigorously in the standard Barraud cages in the laboratory for 1½ years. No fertile eggs resulted from 23 female A. aegypti force-mated with A. albopictus. Further attempts to cross the two species were abandoned in view of the more intensive studies on this cross undertaken by LEAHY (1960).

2. A. SIMPSONI x A. ALBOPICTUS

Reciprocal force-matings between A. simpsoni and A. albopictus achieved no obvious insemination, and no fertile eggs were laid by any of the 28 female A. simpsoni or 14 A. albopictus used.

3. A. AEGYPTI x A. METALLICUS

4 males and 2 females of A. metallicus (Edw.) (Plate XIV d) were obtained together with A. aegypti from eggs wild-collected in Kenya. A single female was successfully force-mated and gave rise to a further 4 laboratory generations by single pair brother-sister matings. Expansion proved impossible through decline in fertility and the line died out. 11 females out of 13 were successfully inseminated by A. aegypti males, but none of the 4 batches of eggs laid was fertile.

4. A. SIMPSONI x A. METALLICUS

20 apparently successful inseminations of female A. simpsoni by male A. metallicus and 9 of the reciprocal cross gave negative results, although egg production was almost normal.

5. A. SIMPSONI x A. APICOARGENTEUS

All endeavours to colonize A. apicoargenteus (Theo.) (Plate XIVc) at the Entebbe laboratory in Uganda had failed

(WOODALL, 1959), so that force-mating was tried at once on the material received from Entebbe with equally disappointing results. Only 4 or 5 males out of more than 50 used achieved proper copulation, giving 2 fertile egg batches and fewer F_1 adults than the original batch. The A. apicoargenteus males seemed hyperactive, reacting violently to contact with the female by rapid abdominal inflexion. An F_2 could not be obtained.

All 10 attempts to force-mate male A. apicoargenteus to A. simpsoni failed, the males showing no copulatory response. A single female A. apicoargenteus, out of 6 tried, was apparently inseminated in the reciprocal cross but without result.

6. A. SIMPSONI x A. DEBOERI

Several egg batches of A. deboeri Edw. (Plate XIV b) were received. The adults responded well to the force-mating technique, but were induced to feed and oviposit only with the greatest of difficulty. Although an F_3 generation was achieved by force-mating, these were insufficient for any further attempts at strain maintenance. Only 2 male A. deboeri were force-mated to A. simpsoni; the eggs laid failed to hatch.

7. A. SIMPSONI x A. MASCARENSIS

14 attempts to cross female A. simpsoni with male A. mascarensis and 4 of the reciprocal cross were entirely

unsuccessful.

8. A. WOODI x A. AEGYPTI

6 out of 7 female A. aegypti force-mated with males of A. woodi were apparently inseminated and 3 laid infertile eggs. In the reciprocal cross all 10 A. woodi females tried were inseminated and laid normal-sized but infertile egg batches.

GYNANDROMORPHS

Ten examples of gynandromorphs have been observed during the course of the present study. These are summarized, together with all known earlier records of mosquito gynandromorphs, in Table XXIX. Their descriptions are briefly as follows :

1. Strain GA. Left palp like shorter ♂ palp, right like longer ♀ palp, left antenna normal ♂, right antenna normal ♀, left wing shorter than right, left side of abdomen shorter than right, external genitalia normal ♂.

2. Strain GA (not related closely to (1)). Left palps and antennae normal ♂, right as normal ♀, left wing shorter than right, left side of abdomen shorter than right, external genitalia normal ♂.

3. Strain CN. Left palp normal ♂, right palp intermediate, left antenna normal ♀, right as normal ♂, left wing normal ♀, right as normal ♂, left side of abdomen colour grade K3 as ♀, right side colour grade J3 as ♂, and shorter, external genitalia ♀.

4. Strain GA/re F3 after outcross to BLTS. Left palp

TABLE XXIX

AUTHOR AND SPECIES		ANT/POST	BILATERAL	OTHER	INTERSEX
FELT (1904)	Aedes abserratus		1		
FELT (1905)	Aedes pullatus			1	
BEDFORD (1914)	Culex theileri		1		
EDWARDS (1917)	Aedes punctor			3	
MARTINI (1921), BRELJE (1923)	A. punctor				1
SHUTE (1926)	A. punctor	1			
MARTINI (1930)	Aedes aegypti	1			
MARSHALL (1938)	Aedes detritus			1	
	Culex pipiens pipiens			1 ?	
	Culex pipiens molestus			1	
WEYER (1938)	C. p. pipiens	1 ?			
CLASSEY (1942)	Culiseta annulata	1			
SMYLY (1942)	A. aegypti			1	
MIDDLEKAUFF (1944)	Culex pipiens fatigans	1		1	
RINGS (1946)	Culex nigripalpus	1			
GILCHRIST & HALDANE (1947)	C. p. molestus		3		
WARREN & HILL (1947)	C. nigripalpus	1			
CARPENTER (1948)	Aedes canadensis	1			
KOMP & BATES (1948)	Culex coronator		1		
	Haemagogus spegazzinii		1		
ROTH (1948)	Culex salinarius	2			
	Orthopodomyia signifera	1			
	Orthopodomyia fascipes			1	
MUSPRATT (1951)	Toxorhynchites brevipalpis		1		
BLASQUEZ & MAIER (1951)	C. p. fatigans				50
ROTH & WILLIS (1952)	A. aegypti	1		2	
GRATZ (1954)	C. p. molestus		1		
LAVEN (1955a)	C. p. molestus				++
(1957a)	C. p. molestus	·····57·····			
LAURENCE (1959)	Mansonia uniformis				1
ANTUNES & FORATINI (1960)	A. aegypti	1		2	
PATERSON & BROOKEWORTH (1961)	Aedes pembaensis	1			
HORSEFALL & ANDERSON (1961)	Aedes stimulans				++
VANDEHEY & CRAIG (1961)	A. aegypti	·····80·····			
McCLELLAND (present study)	A. aegypti	3	4	2	3
	Aedes deboeri		1		
TOTALS	31 references - 7 genera - 22 species	17	14	16	5

TABLE XXIX. Gynandromorphs and intersexes recorded in the literature and in the present study.

NOTES A large unstated number is entered as ++ . These and the other three large numbers have been omitted from the totals of individual.

normal ♂, right intermediate, both antenna ♂, remainder ♀ (Plate XVII a).

5. Same family as (4). Both palps normal ♀, both antennae ♂, remainder ♀ (Plate XVII b).

6. Same family as (4). Left palp ♀, right ♂, both antenna ♂, remainder ♀ (Plate XVII c).

7. Same family as (4). Left palp normal ♀, right as modified ♂, both antennae ♀, remainder ♂ (Plate XVII d).

8. Same family as (4). Both palps and both antennae normal ♀, remainder ♂ (Plate XVII e). This gynandromorph took a blood meal but had excreted most of the blood apparently unaltered within 24 hours.

9. Same family as (4). Left palp ♂, right as ♀, left antenna ♂, right as ♀, left wing ♂, right as ♀, left side of body shorter than right, external genitalia ♂ (Plate XVII f).

10. Aedes deboeri from egg laid by wild-caught female. Left palp ♀, right as ♂, left antenna ♀, right as ♂, left wing longer than right, left side of body longer than right, external genitalia ♂.

INTERSEXES

Three apparent intersexes appeared in an F_6 A. mascarensis/aegypti hybrid family. Resembling males in general they showed cephalic and genital abnormalities.

It was the withered and bedraggled appearance of the palps and antennae which first attracted attention (Plate XVIII g,h,i.k.). The terminalia of all three showed, in cleared preparations, exaggerated development of what are presumably the apical paraprocts (1) into large lobes and a great reduction of the claspettes (2) and the associated spines (Plate XIX m,n.o.). The appearance in life (Plate XVIII j) suggested the combination of both male and female genitalia (c.f. LAURENCE, 1959). In one example a single spermatheca (3) is clearly visible (Plate XIX o). The other two specimens however were the only ones dissected. In neither were ovaries nor spermathecae visible, while apparently normal testes were present in one and absent altogether in the other. More detailed examination of this material has been deferred.

DISCUSSION

HYBRIDIZATION STUDIES

The production of fertile hybrids between two species by forced-mating or any other laboratory method probably indicates that they are sufficiently closely related to have highly homologous chromosomes. Unless hybridization can be demonstrated under natural conditions, no doubt need be shed on the validity of either species, since chromosomal incompatibility must usually be the ultimate consequence, rather than a cause, of genetic isolation between populations. A. aegypti and A. mascarensis seems to be a case of allopatric speciation in process of being broken down following the overlap of the two populations. The situation in Mauritius certainly deserves further study, so little seems really known of the bionomics and distribution of A. mascarensis.

A. woodi and A. simpsoni are sympatric species, but here it is likely that isolation is behavioural. The failure to obtain hybrids between female A. aegypti and A. simpsoni is of doubtful significance in view of the small numbers tried.

Similarly none of the negative evidence in the other 8 crosses precludes the possibility of successful hybridization (c.f. the single hybrid obtained from 50,000 eggs by WOODHILL, 1959), but it nevertheless suggests that the chance of hybridization between the species paired is less than in the three successful crosses.

It is to be hoped that improvement in the techniques of preparing polytene chromosomes will enable the banding patterns to be mapped, not only in A. aegypti but in other species of Stegomyia as well, for comparison with the hybrids. The extent of similarity between the chromosomes of A. aegypti and A. mascarensis would be particularly revealing. The relative difficulty in obtaining hybrids between A. aegypti and A. simpsoni and their apparent sterility might then be explicable in terms of lack of homology.

It is possible that A. mascarensis can be regarded as a multiple recessive of A. aegypti, in which case crossing and backcrossing would yield valuable linkage information. The preliminary results suggest that ks at least can function as an efficient marker gene in A. aegypti and there is an indication that it may be partially sex-linked. The vigour of the ks in A. aegypti strain has improved during the year; it has been colonized but no attempt has yet been made to use it. If all the mascarensis genes can function when isolated in the aegypti genome, A. mascarensis is a wonderful store of "ready-made" mutants.

GYNANDROMORPHS AND INTERSEXES

With the realization that both gynandromorphs and intersexes can be genetically determined, their occurrence and morphology per se is of less interest than the mechanism of their production. The isolation of strains consistently

producing them, as LAVEN (1955a, 1957a) has done for C. p. molestus, is a prerequisite of further useful work. The most remarkable fact is that no gynandromorph or intersex has ever been reported for Anopheles despite the enormous numbers that must have been examined by malariologists. The explanation may involve the presence in Anopheles of distinct small heterosomes, yet gynandromorphs are reported from a wide range of other insects which resemble Anopheles in karyotype more closely than do the Culicines.

FINAL REMARKS

Points for future research have been suggested above where appropriate. It remains to emphasize that there is still a great need for the isolation of further good marker genes and linkage measurements. The possible mascarensis genes apart, the store of naturally occurring mutants in A. aegypti seems by no means exhausted; much that has been passed-over or has eluded isolation can be expected to be seen again. When there is a need of irradiation-induced mutants, the use of phosphorus^{32} in the larval rearing medium suggested in the introduction should be worth a trial.

The whole area of mosquito genetics is widening and A. aegypti is proving to be the most attractive species for basic study. The present work was deliberately planned to be broad in scope and therefore suffers from a certain incompleteness and lack of conclusion. It must therefore be accepted as an interim report, a consolidation of work done and a base from which future research can be planned.

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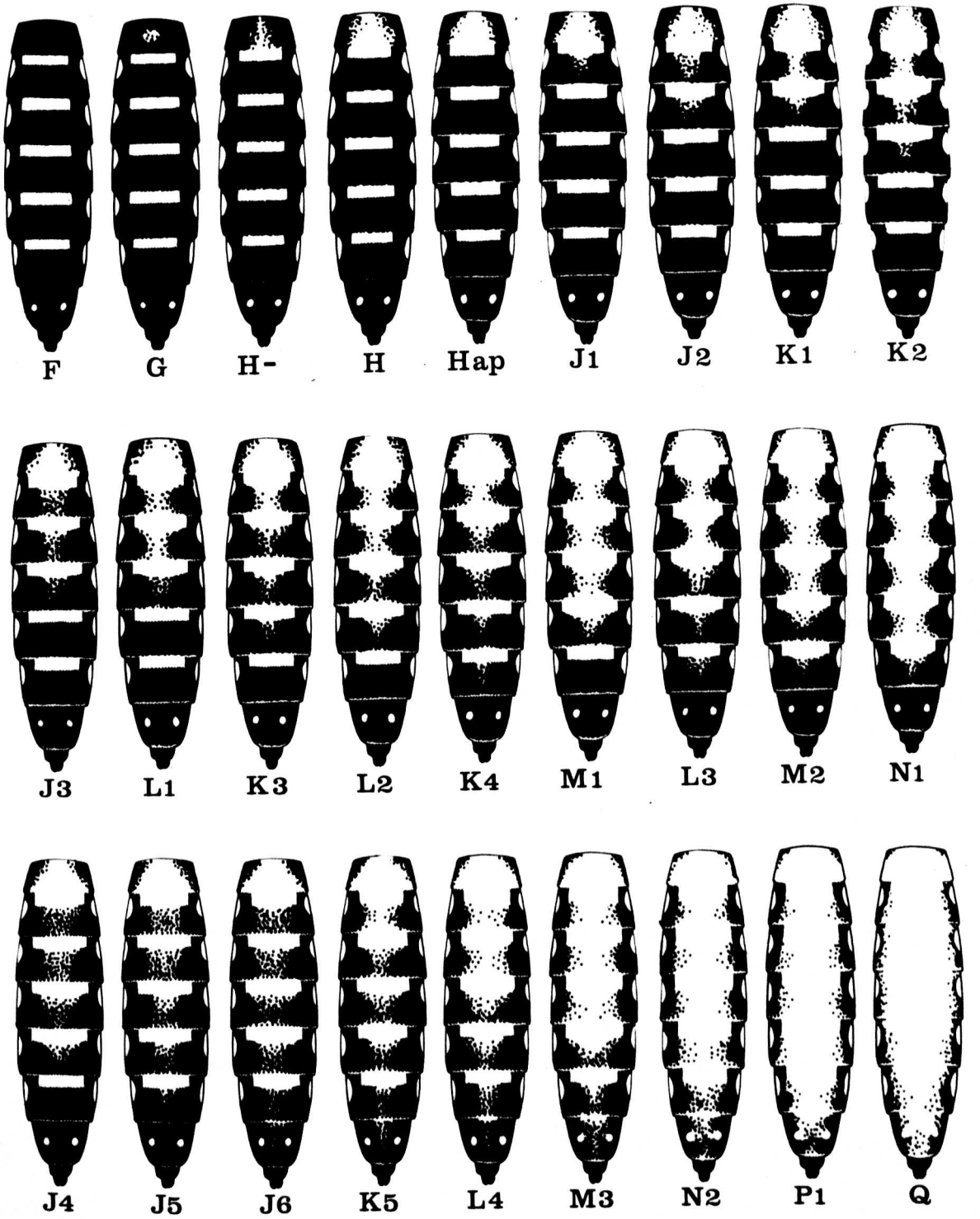
PAGE NUMBERING AS ORIGINAL

PLATE I

Key to abdominal colour grades (Schematic). Of the 37 the following 10 have been omitted: G-, H-ap, KO, LO, MO, NO, PO, Q-, Q+ and R.

PLATE I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J5
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L4
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N2
P1
Q

PLATES II - IV

Techniques.

(a.) Bowl with perspex cover and label used for rearing larvae.

(b.) Brass plunger used for tamping down cotton wool in shell vials. (1) bypass hole (2) perforated bottom plate.

(c.) Wire rack holding shell vials of pupae plugged with coloured cotton wool.

(d.) Wire rack holding single-pair paper cup cages on oviposition vials.

(e.) Binocular set-up for examining adults (1) lint-lined dish (2) foot focussing attachment (3) plasticine surface (4) black perspex stage.

(f.) Monocular set-up for photography (5) universal mounting on extension arm (6) camera adaptor (7) perspex photographic stage with central suction-hole (8) lamps.

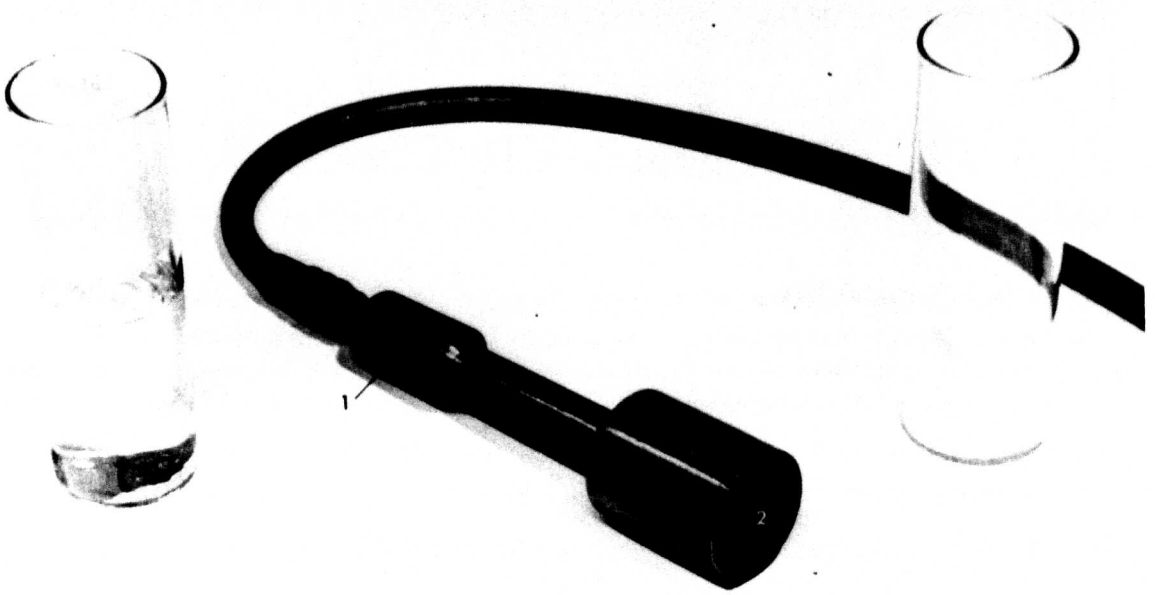
(g.) Barraud-type mosquito cage with "Ogden" machine resting on top (9) thermostat (10) heater (11) stirrer (12) record label (13) cage frame.

(h.) Preparation of male A. albopictus for hand-mating.

(i.) Applying anaesthetized female to prepared males glued to perspex disc.

PLATE II

a



b

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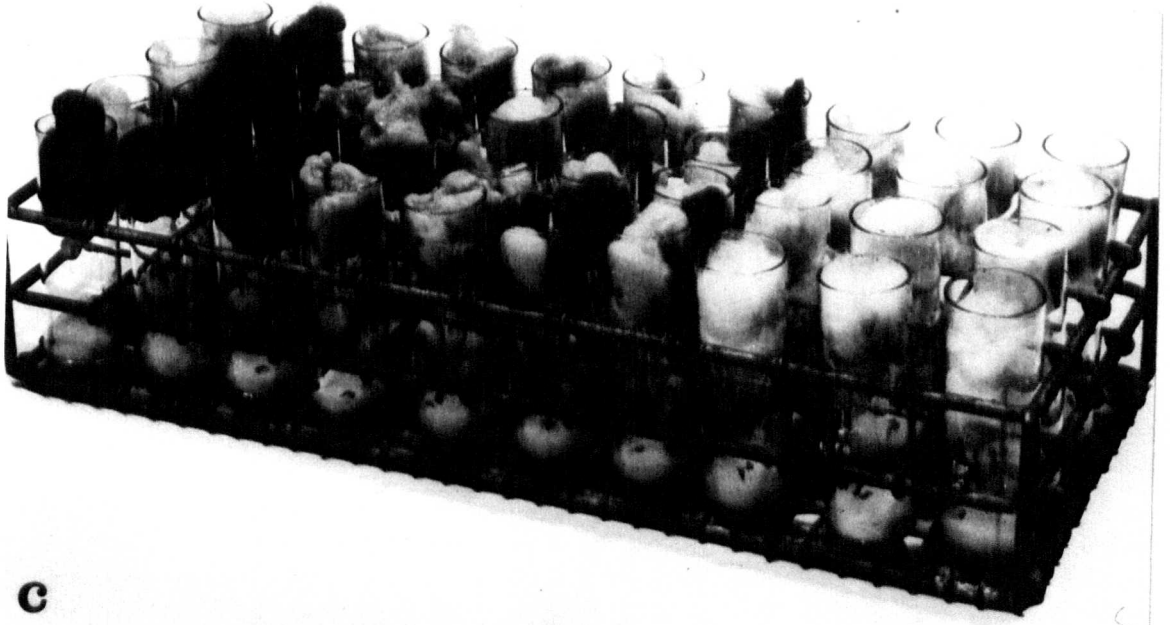

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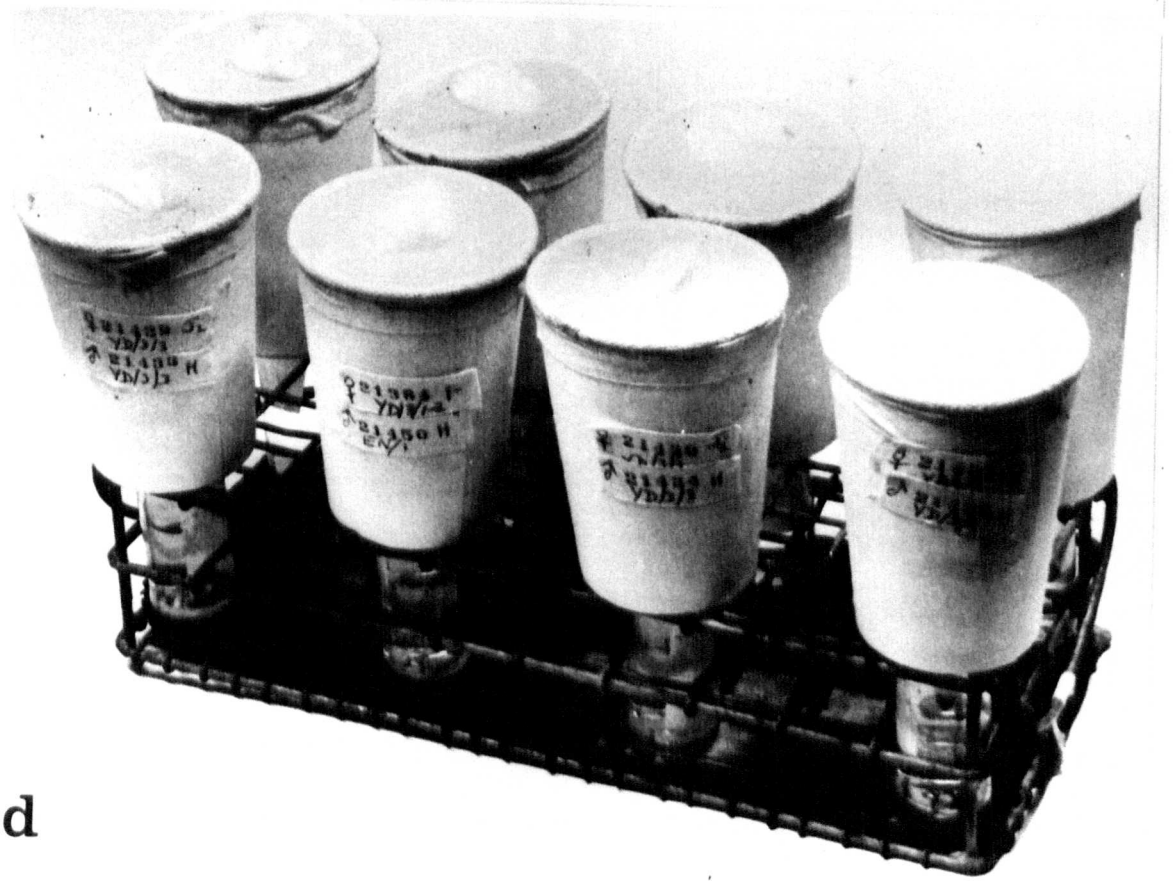
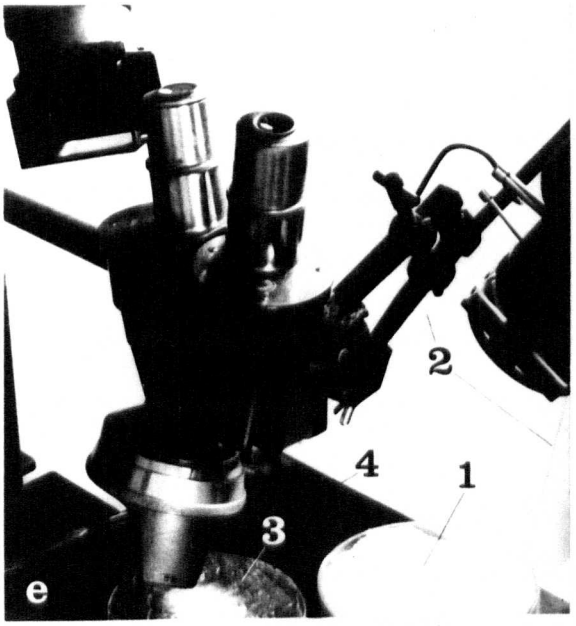
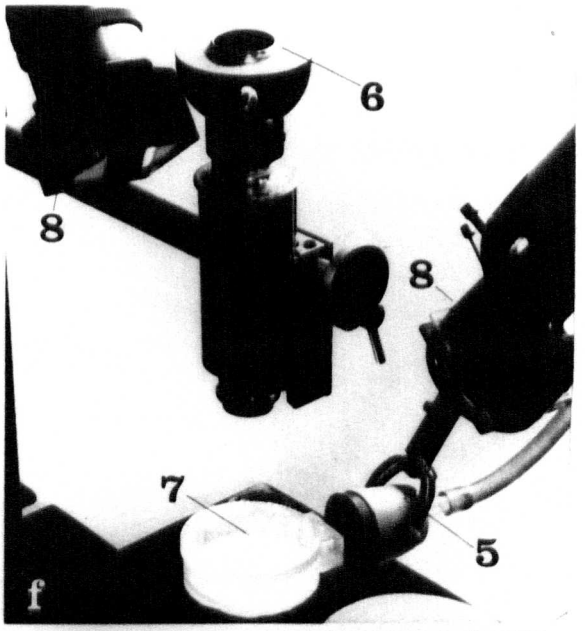
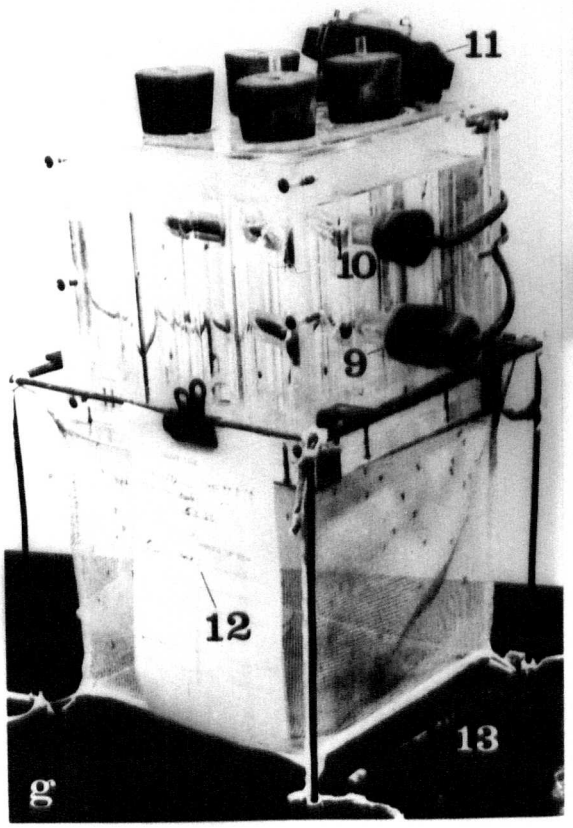


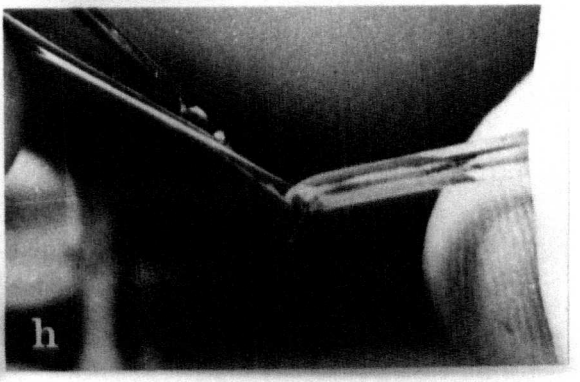
d

PLATE IV









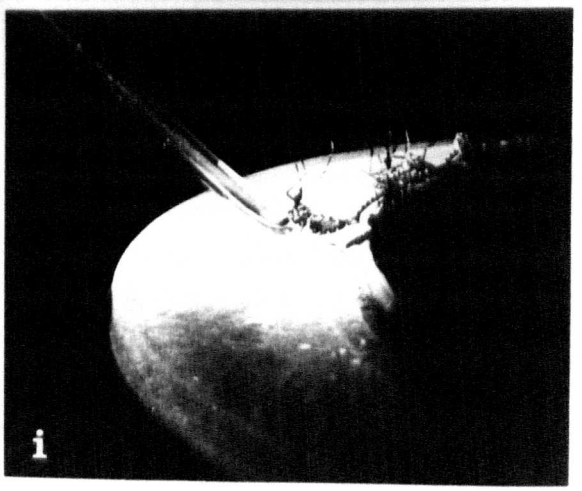


PLATE V

Eye colours in *A. aegypti*

(a-g) etherized males viewed in air.

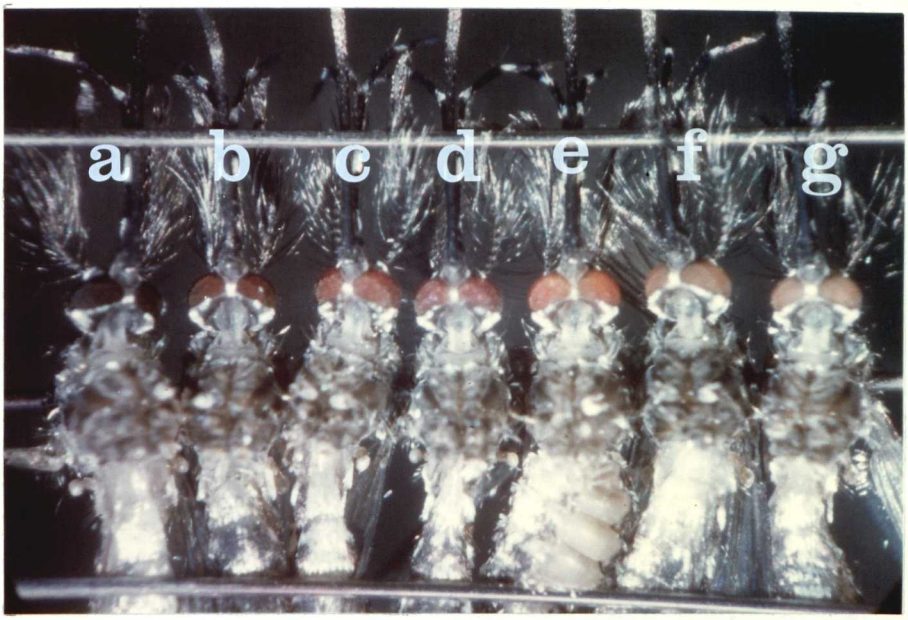
(h-n) the same males cleared in xylol and mounted in DePeX.

(a/h) wild-type (b/i) *ol*.

(c/j) *ru* (d/k) *re* (e/l) *ru re*

(f/m) *ol ru* or *ol re* (g/n) *ol re ru*.

PLATE V



PLATES VI & VII

pa alleles and crosses.

(a) PR pa ♀

(b) PR pa ♀ intermediate

(c) GA pa ♀ showing assymetry

(d) GA pa ♀

(e) PR pa ♀ sternites

(f) YD formosus ♀ sternites

(g) PR pa ♂

(h) GA pa ♂

(i) PR pa ♀ intermediate

(j) Cross GA grade G ♀ x PR pa ♂

(k) Cross GA pa grade Q ♀ x GA grade F

(l) Cross CR pa ? ♀ x GA pa ♂

(m) CC pa ? ♀ intermediate

(n) Cross PR pa grade Q ♀ x CC grade H ♂

(o) Cross CC grade M3 ♀ x PR grade J6 ♂

(p) CR pale ♀

PLATE VI

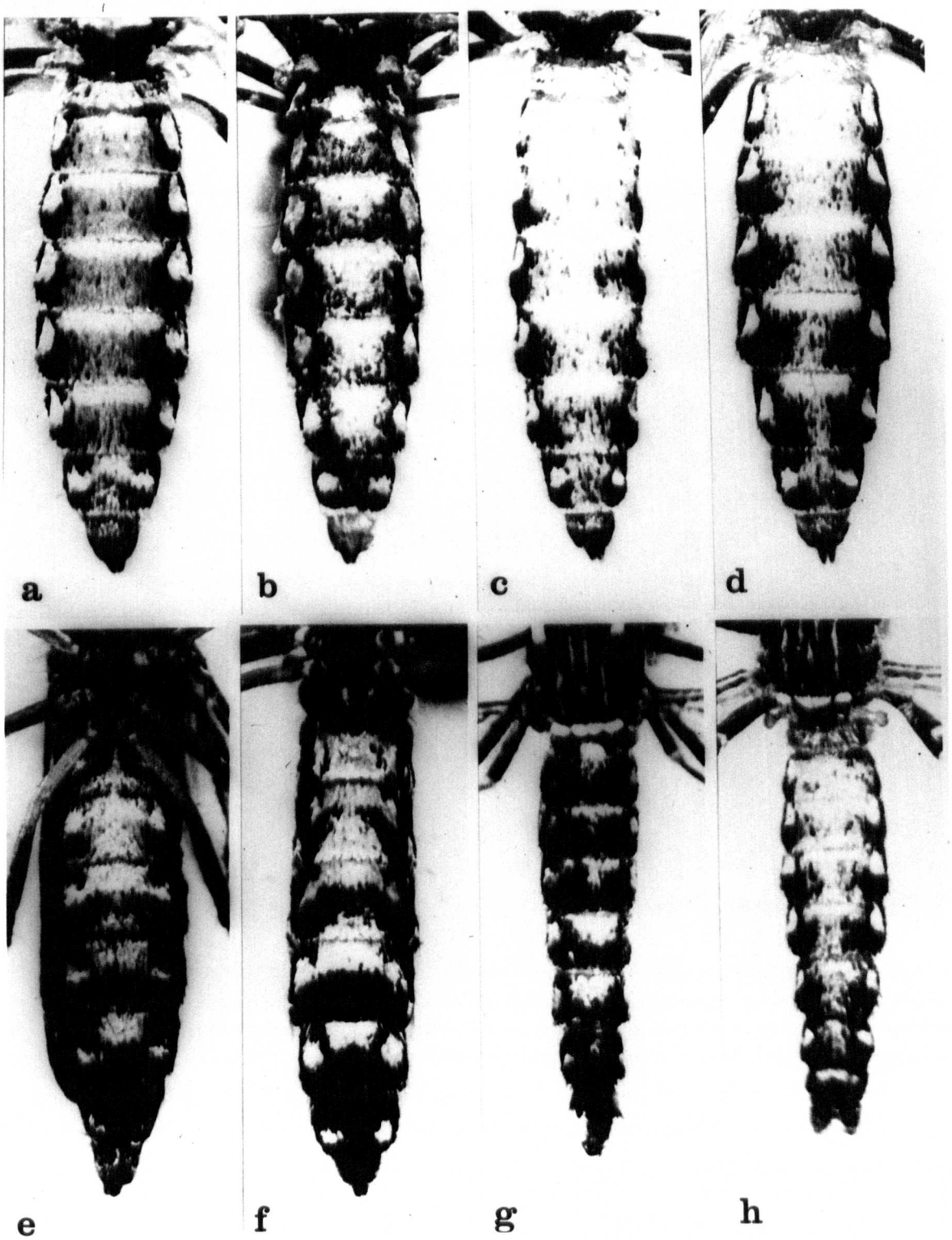
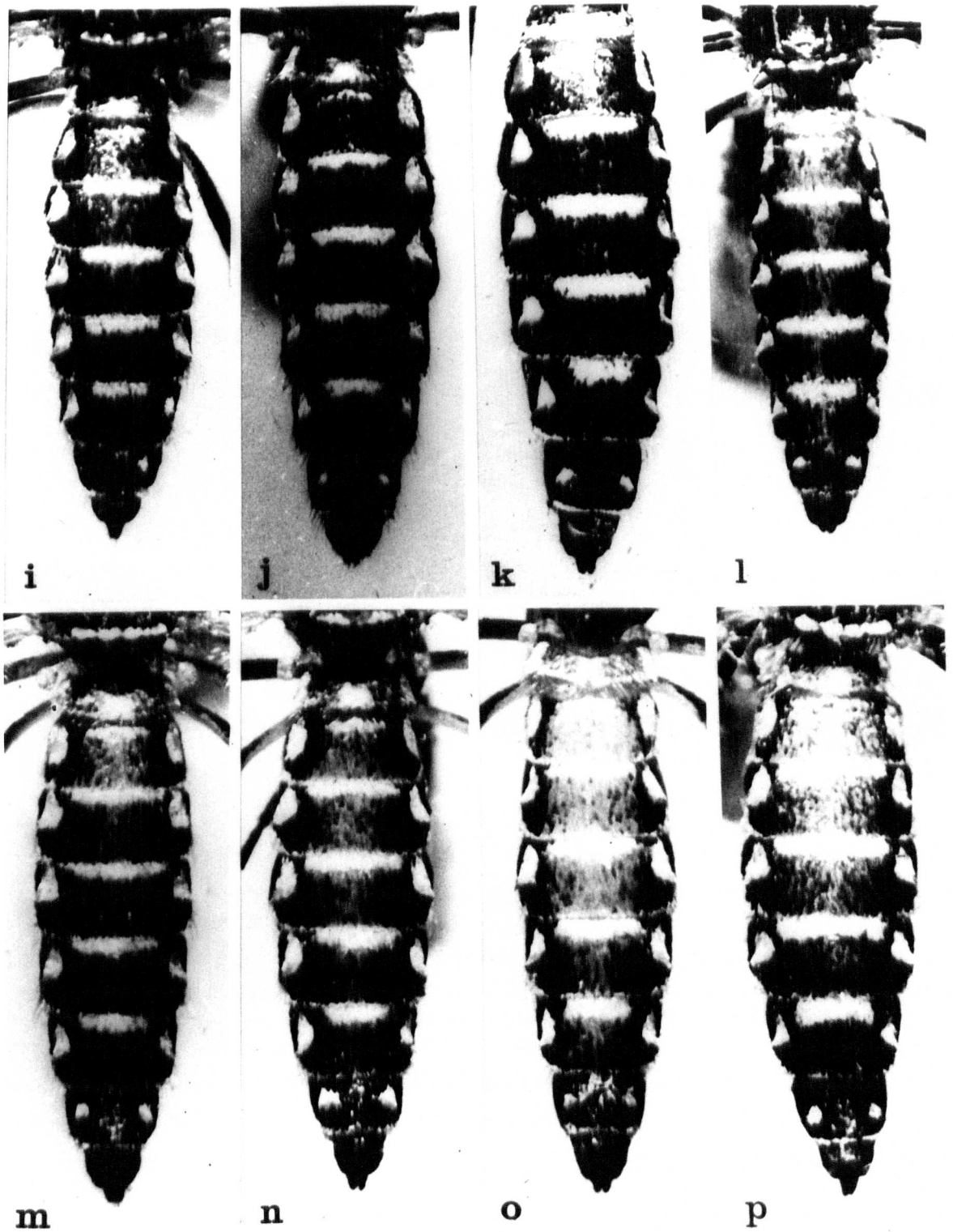


PLATE VII



PLATES VIII - X

s alleles.

(a) JD s^g ♀

(b) DH s^g ♀

(c) PH s^g ♀

(d) GH s^g ♀

(e) DH s^g / s^+ ♀ heterozygote pale

(f) Ditto dark

(g) JD s^g ♂

(h) JD s^g / s^+ ♂ heterozygote

(i) MA s^w ♀

(j) GA s^w ♀

(k) GA s^w ♀

(l) GA s^w ♀

(m) MA s^w / s^+ ♂ heterozygote

(n) MA s^w ♂

(o) GA s^w ♂

(p) GA s^w ♂, basal bands absent.

(q) DK s^r ♀

(r) FS s^p ♀

(s) F_4 ♀ from Cross PR pa x FS s^p

(t) FS s^p ♂

(u) DK s^r ♀ sternites

(v) F_1 ♀ Cross EK s^g x GA s^w

(w) GA s^w / s^+ ♀ heterozygote

(x) GA s^w ♀ basal bands absent.

PLATE VIII

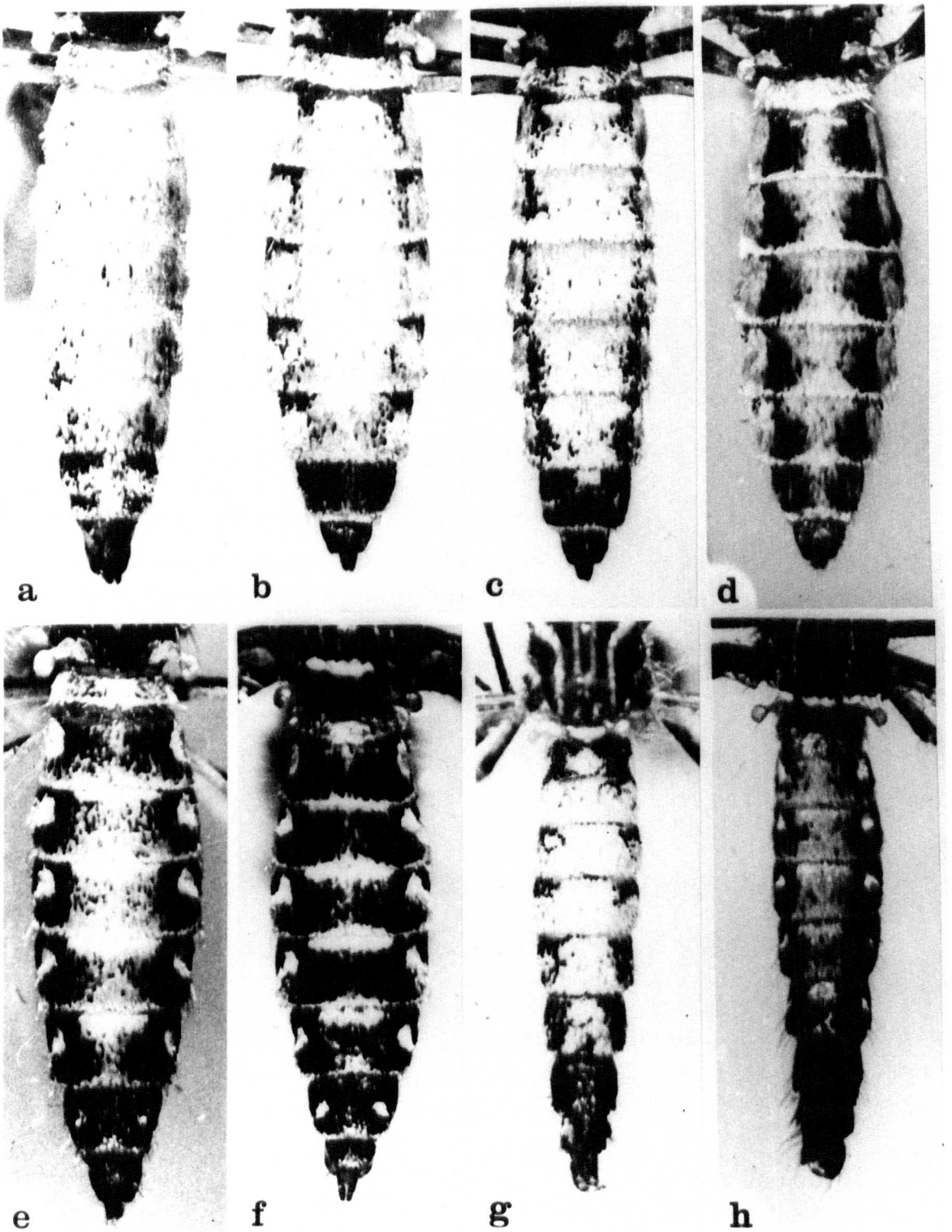


PLATE IX

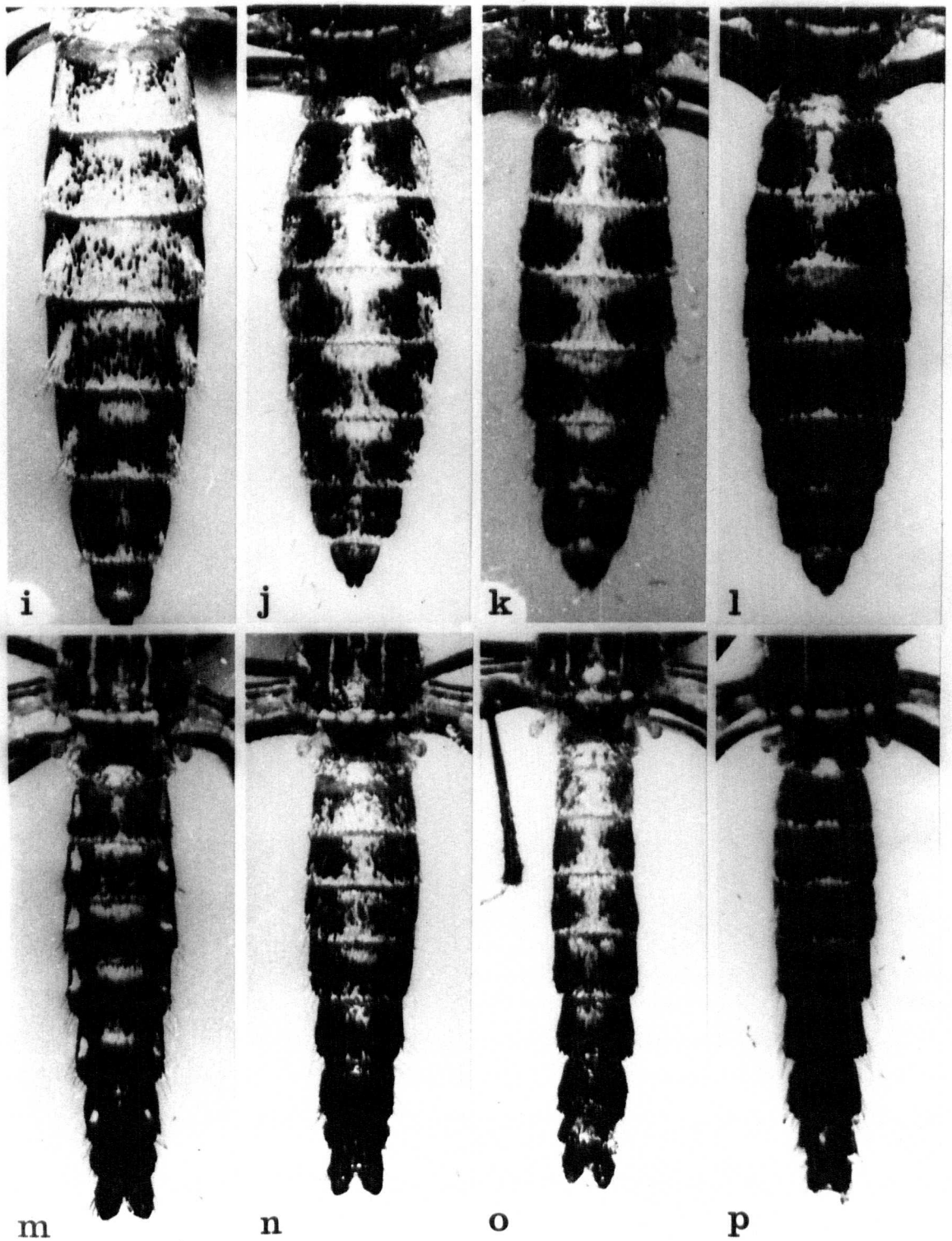


PLATE X

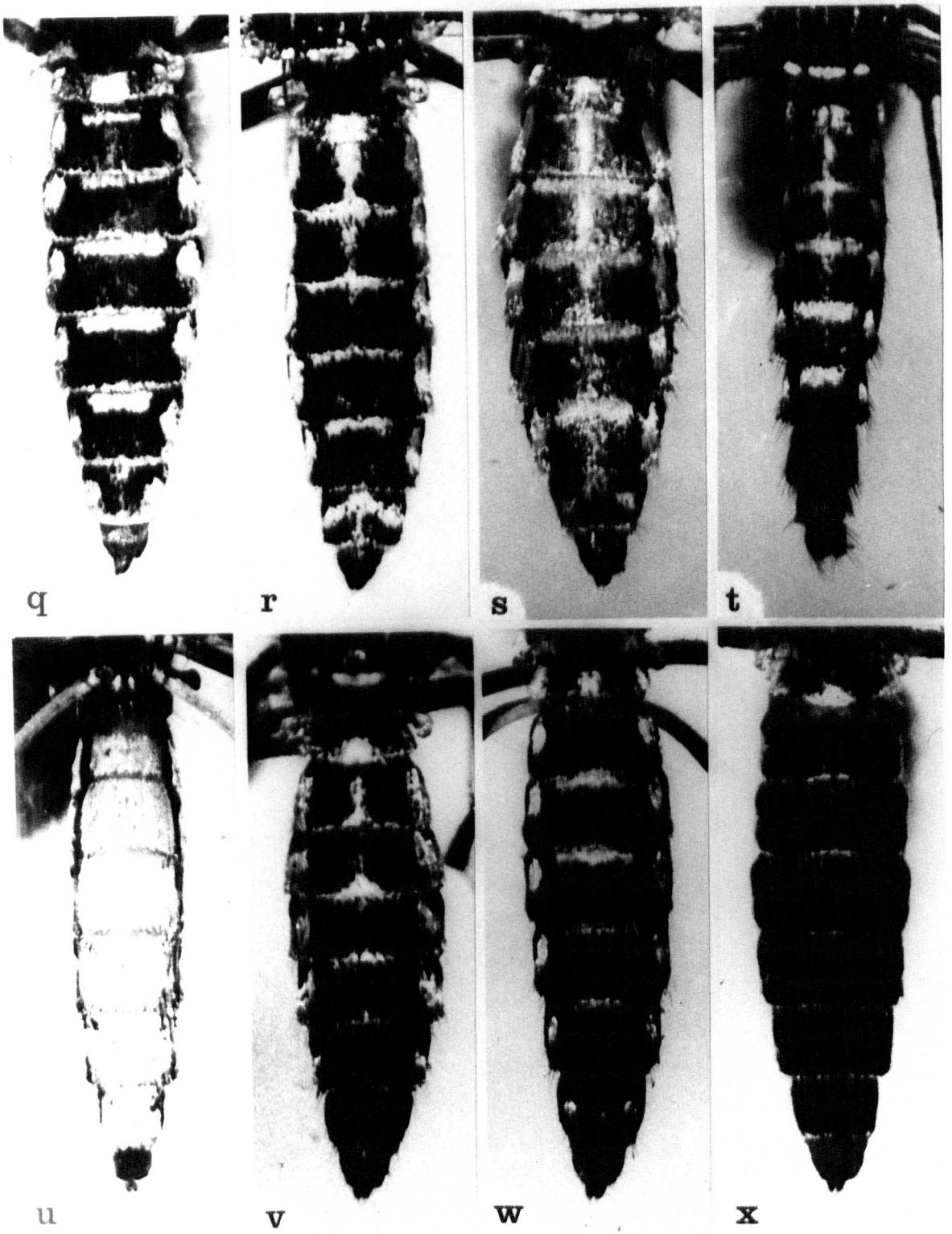


PLATE XI

Miscellaneous forms.

(a) DK wild-type ♀

(b) MI palest ♀

(c) TV palest ♀

(d) SG grade J1 ♀

(e) TA *formosus* ♀

(f) TA *formosus* ♂

(g) TV wild-type ♂

(h) GH basal bandless ♀

PLATE XI

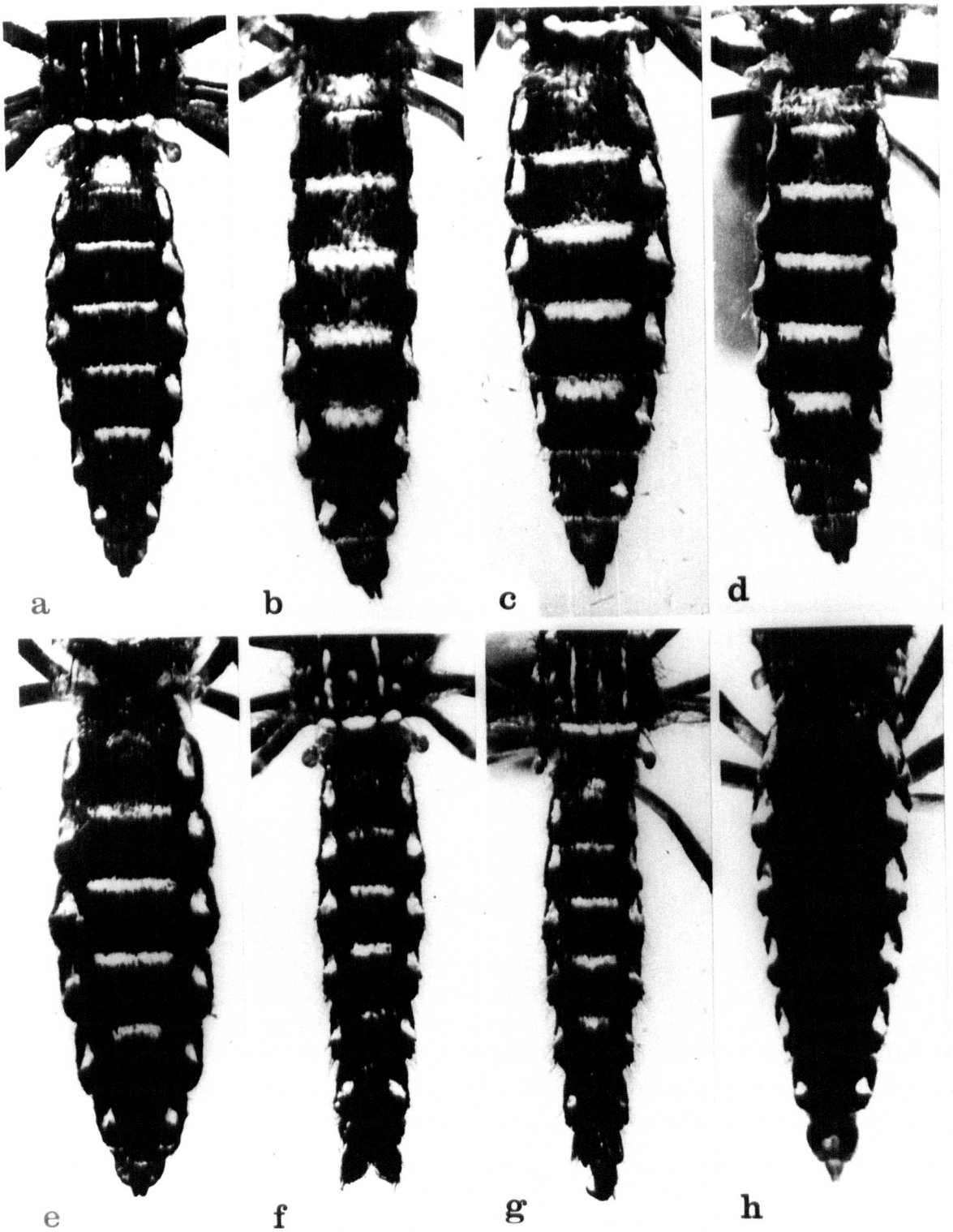


PLATE XII

Metatarsal variants.

(a) *th*

(b) wild-type

(c) *blt*^{2}

(d) *blt*^{1}

(e) "half-black"

(f) *A. simpsoni*

(g) *A. simpsoni* x *A. aegypti blt*$^{+}$

(h) *A. simpsoni* x *A. aegypti blt*^{2}

PLATE XII

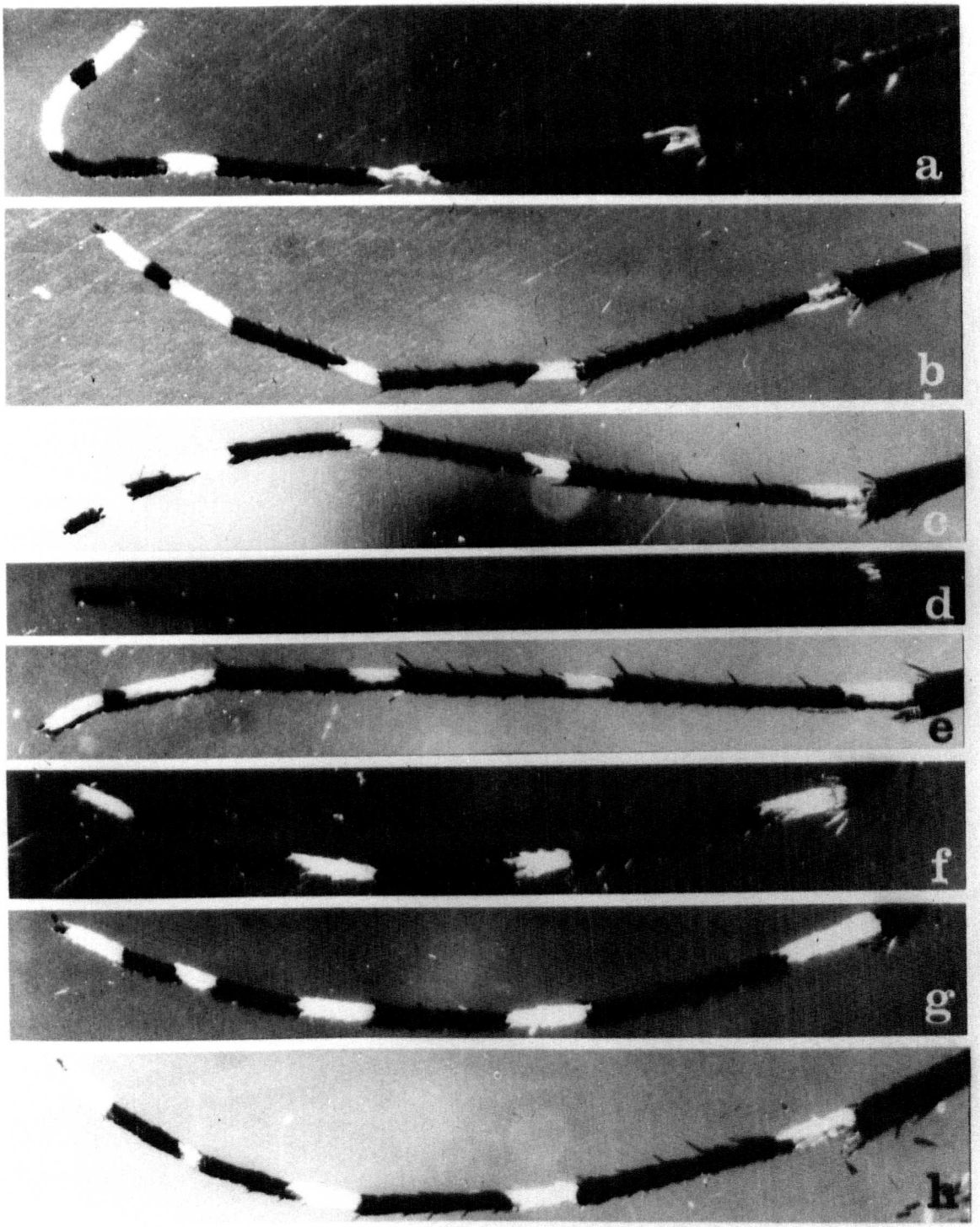


PLATE XIII

Mesonotum mutants (all female).

(a) RB ds sl with black vertex

(b) CN second dark scutum variant

(c) YD first dark scutum variant

(d) YD, median paired lines faint

(e) YD, median paired lines as wild type

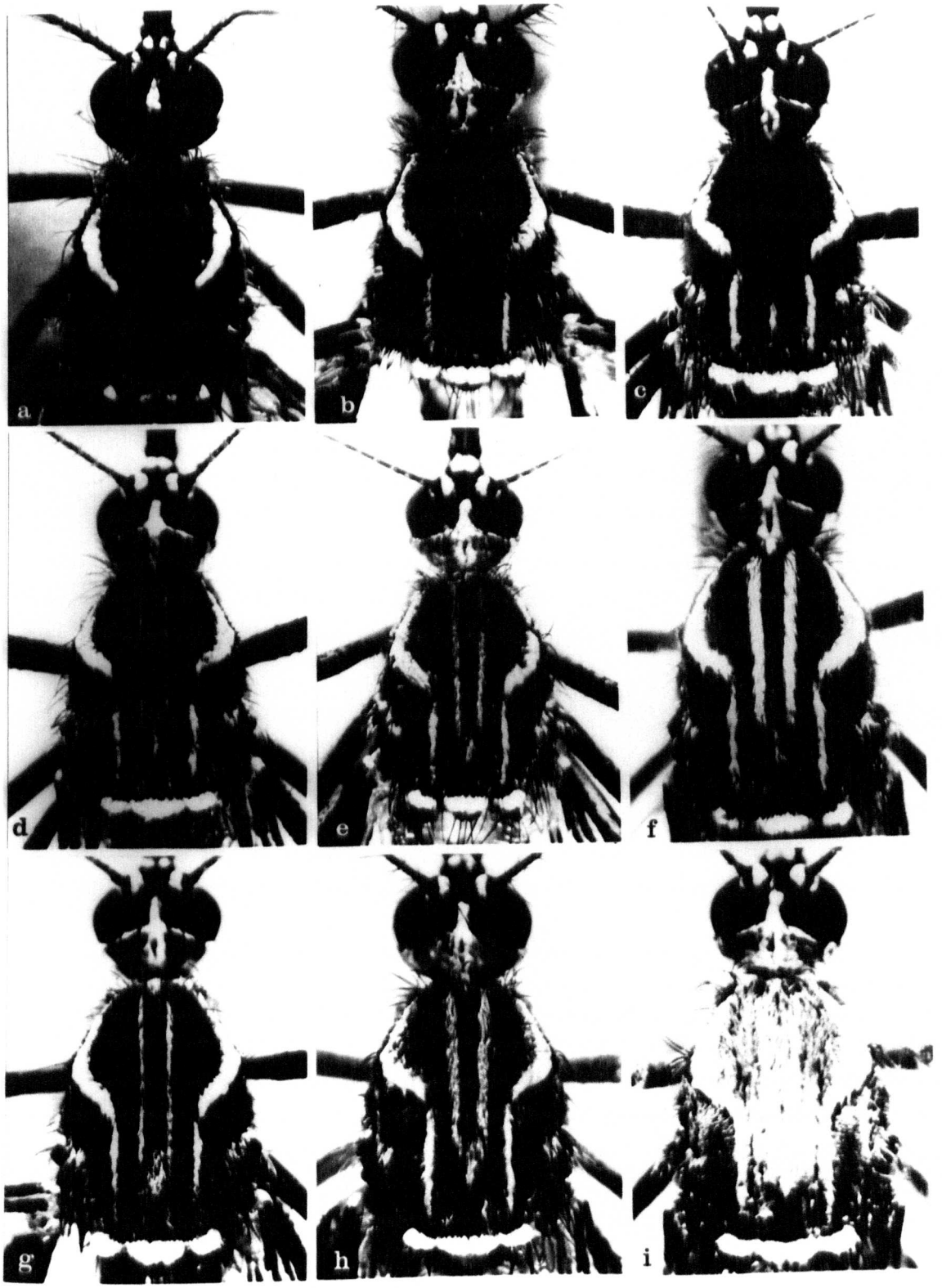
(f) PR St - Stripe, vertex dark.

(g) GH Fl - Fleck.

(h) PR St - Stripe, vertex pale.

(i) KR G - Gold mesonotum.

PLATE XIII



PLATES XIV - XV

The mesonotum in other *Stegomyia* species (All females except n).

(a) *A. albopictus*

(b) *A. deboeri*

(c) *A. apicoargenteus*

(d) *A. metallicus*

(e) *A. simpsoni*

(f) Hybrid, *A. simpsoni* x *A. aegypti*

(g) *A. woodi*

(h) F_1 hybrid, *A. woodi* x *A. simpsoni*

(i) F_2 hybrid, *A. woodi* x *A. simpsoni*

(j) *A. mascarensis* type form

(k), (l) *A. mascarensis* variants from field-collected eggs.

(m) F_1 hybrid *A. aegypti* x *A. mascarensis*

(n) male *A. mascarensis* with single antenna from field-collected egg.

(o) *sl* gene in *A. aegypti*

(p) *A. mascarensis* pt^+ - paratergites white -scaled.

(q) *A. mascarensis pt* - paratergites bare

(r) *A. mascarensis* pt^+ sl^+ from field-collected eggs.

PLATE XIV

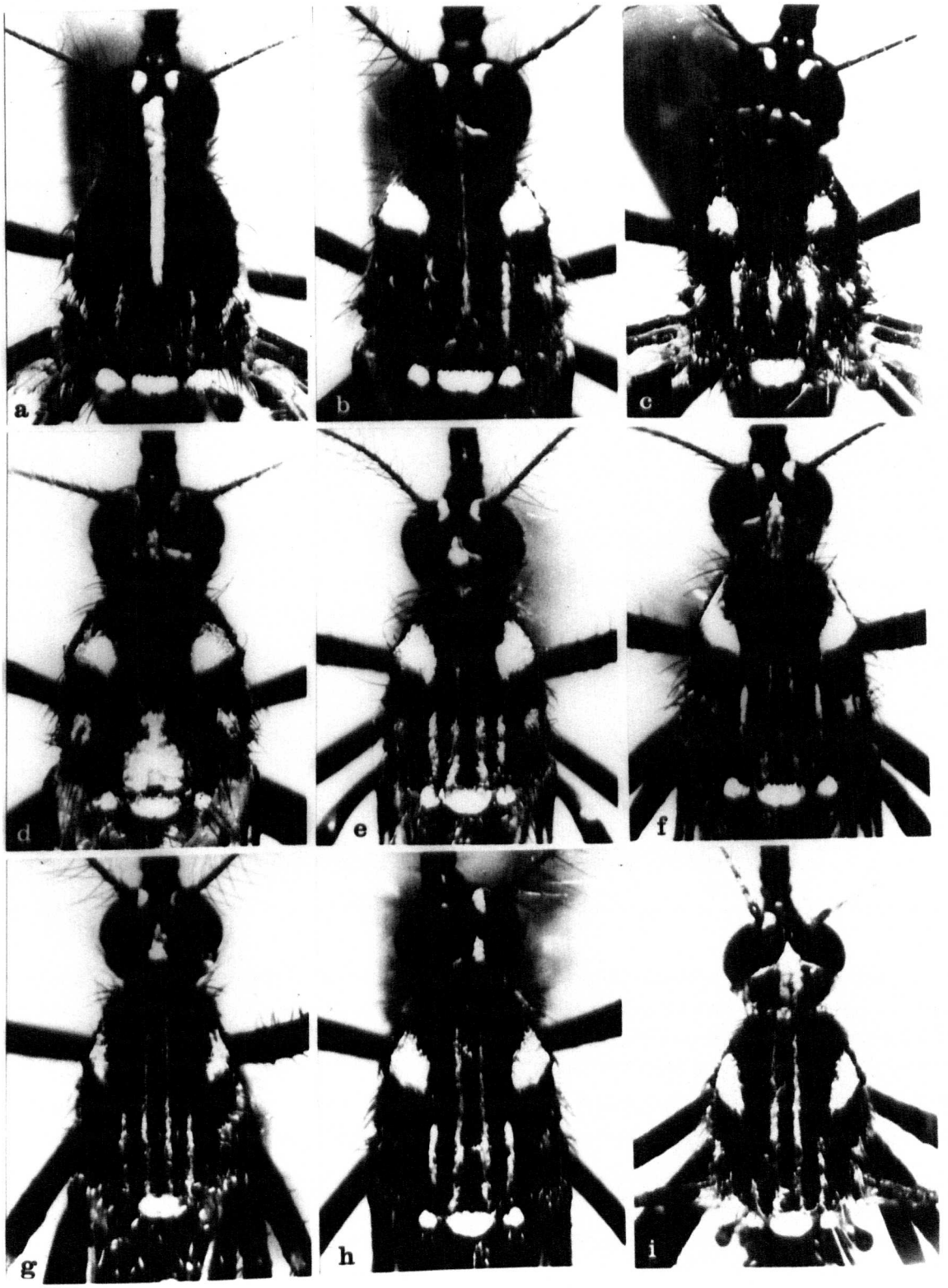


PLATE XV

PLATE XVI

Various aberrations.

(a) Somatic mutation in PR *pa* heterozygote.

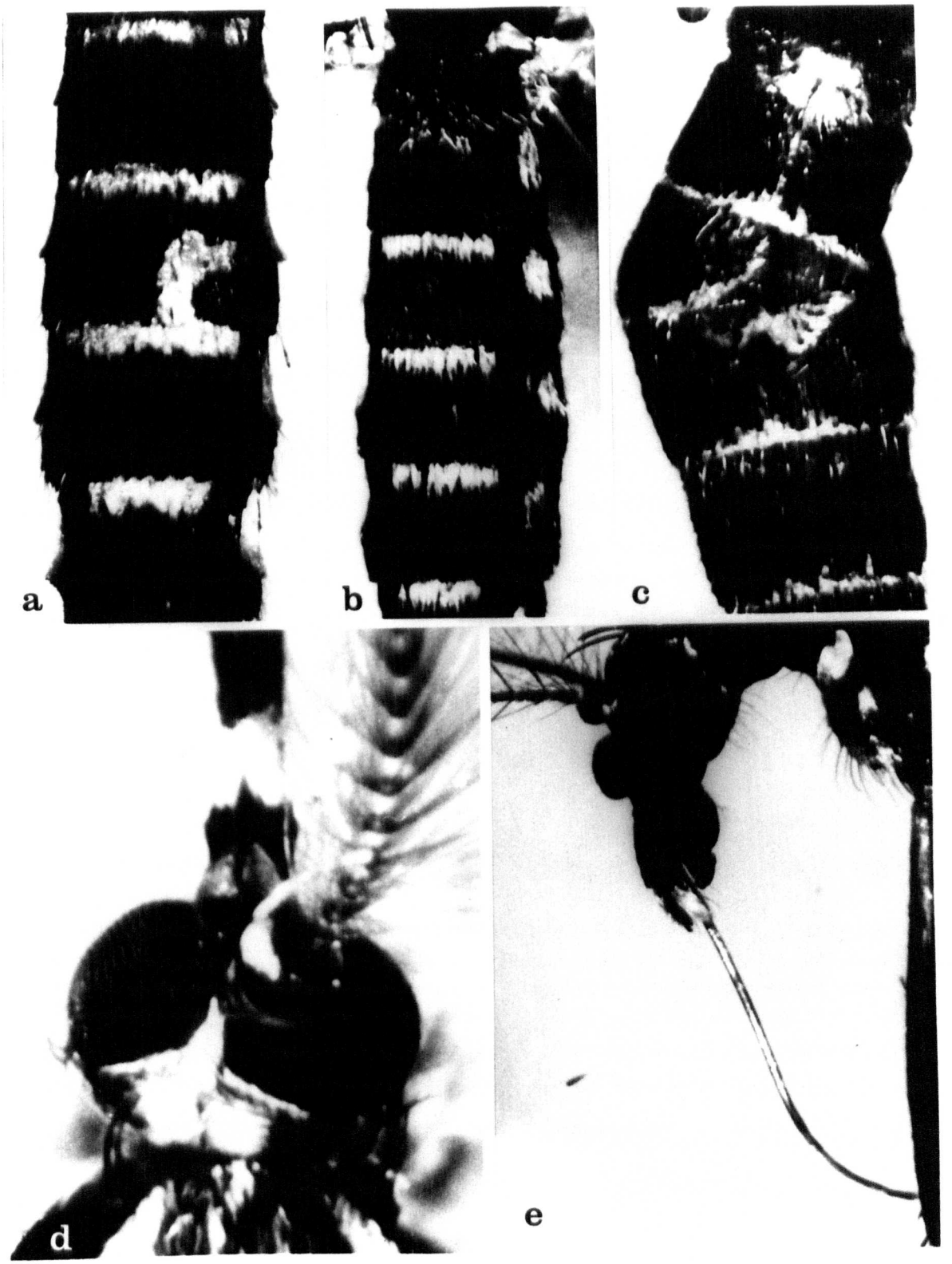
(b) *fz* - fuzzy.

(c) RB - unilateral development of tergite.

(d) *A. mascarensis* with single antenna.

(e) Short labium in *A. simpsoni* x *A. aegypti* hybrid.

PLATE XVI



PLATES XVII - XIX

Gynandromorphs, etc.

(a-f) 6 gynandromorphs from a single family. GA re F3 after outcross to BLTS.

(g-h) heads of two intersexes.

(i,k) whole mosquitos as above.

(j) lateral view showing apparent male and female genitalia combined.

(l) normal ♂ genitalia.

(m-o) genitalia of intersexes
(1) apical paraprocts
(2) claspettes
(3) spermatheca

(p) NJ, abberant basimere.

(q) bent proboscis.

PLATE XVII

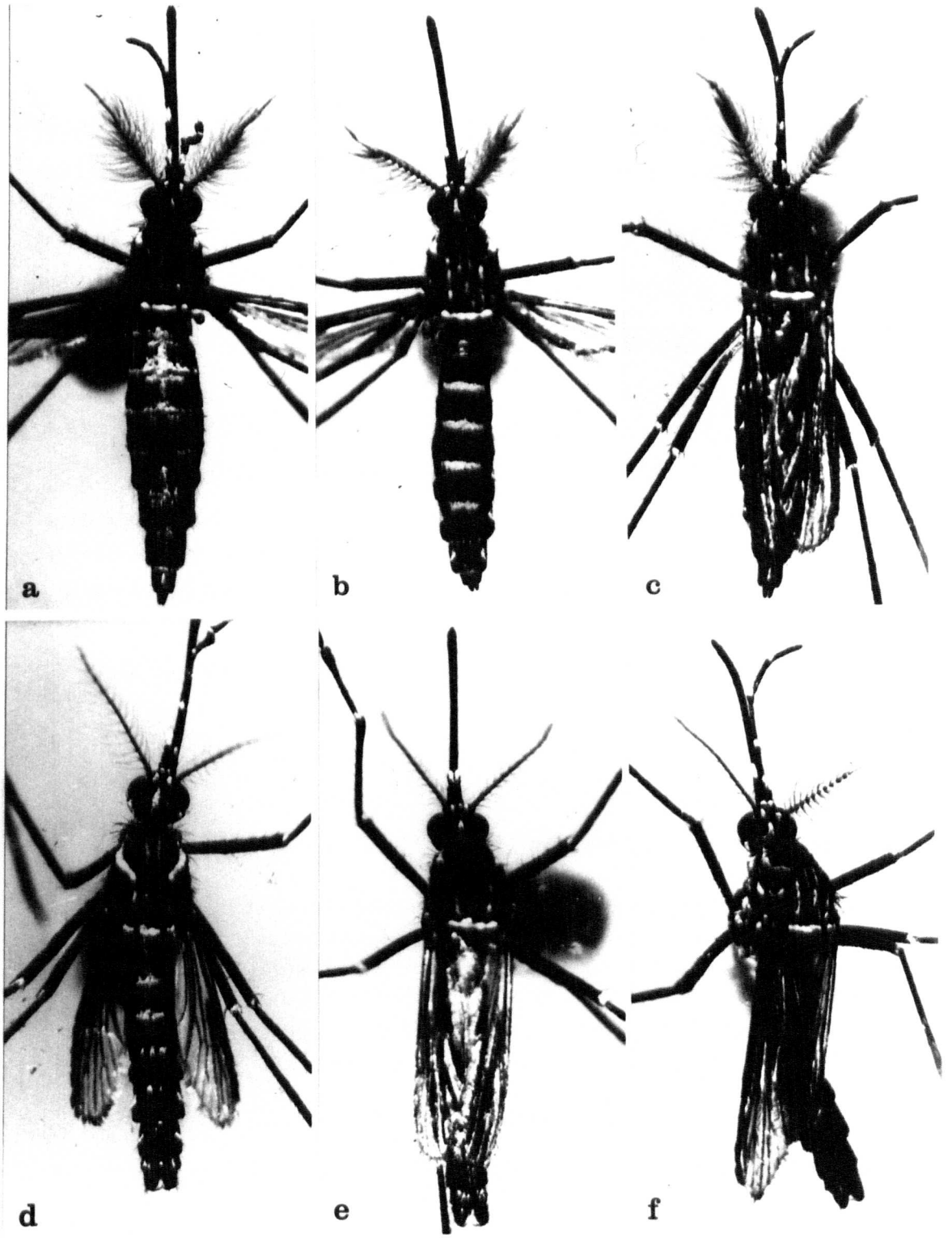


PLATE XVIII

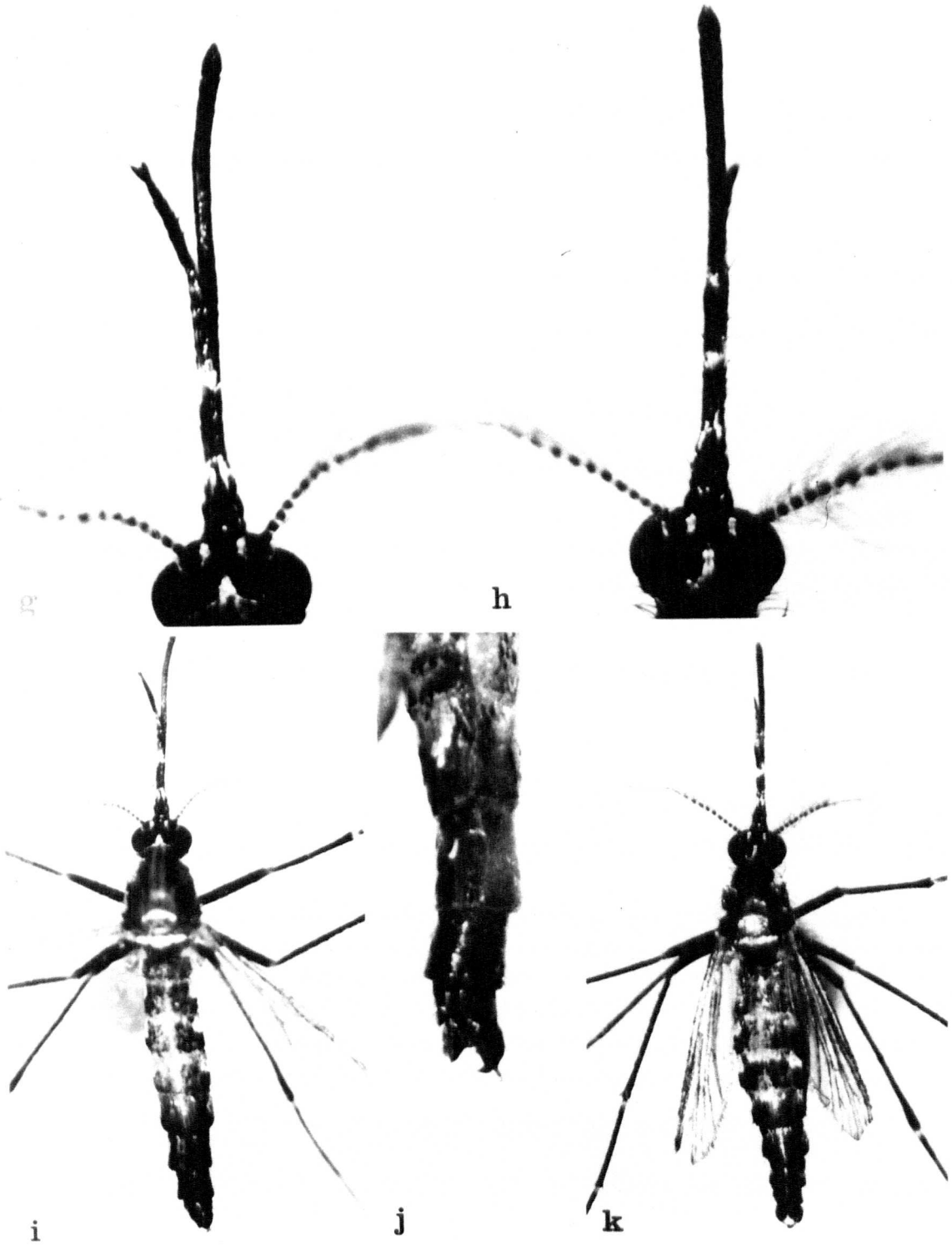
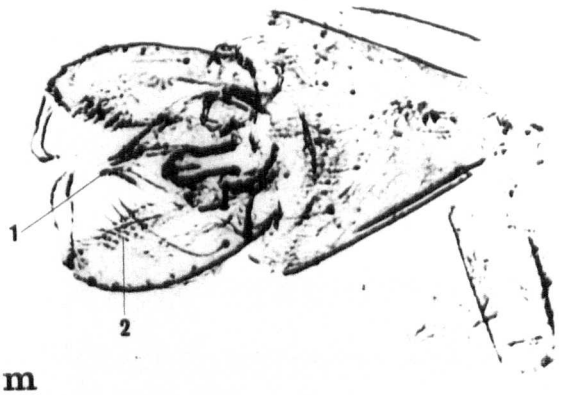
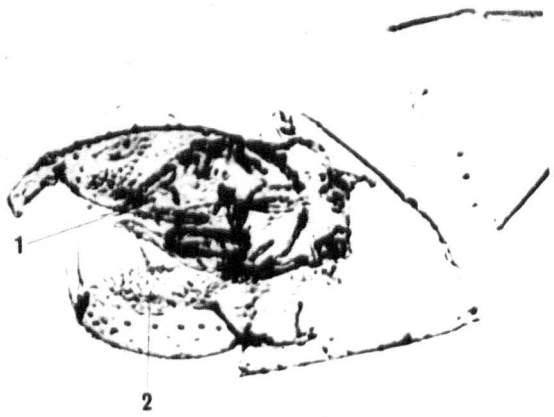


PLATE XIX

l



m



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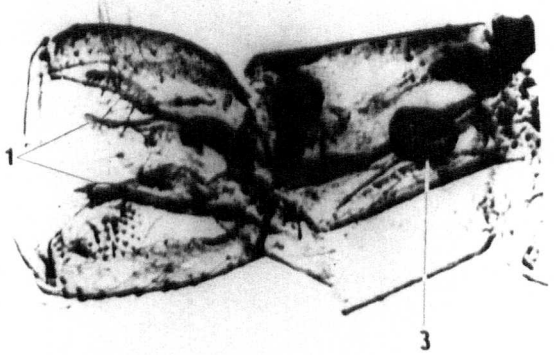
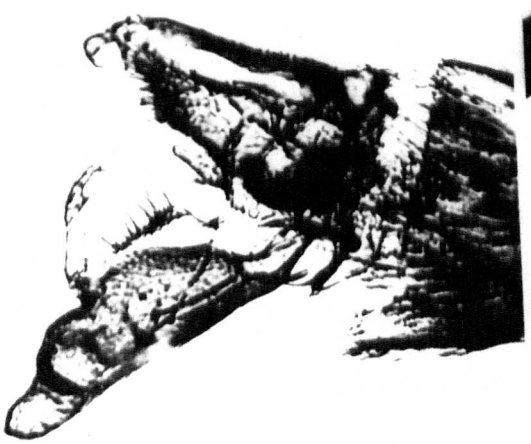
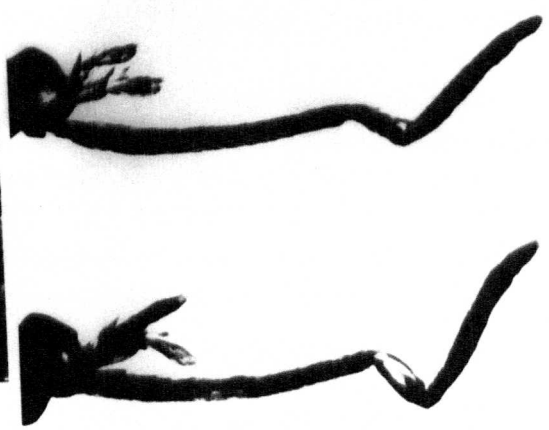


o



p



q