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NOTES AND COMMENTS

A NEW HYPOTHESIS FOR THE ORIGIN OF THE PARTHOGENETIC GRASSHOPPER *MORABA VIRGO*

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SUMMARY

It is proposed that *Moraba virgo*, the only all-female grasshopper, originated by hybridisation between two extant species *P169* and *P196*. Such an origin accounts for *M. virgo*'s peculiar karyotype and pattern of late replication. The model is predictive and experiments are suggested to test it.

1. BACKGROUND

THE Australian morabine grasshopper *Moraba virgo* Key (Orthoptera: Eumastacidae) is the only known thelytokous species in the Acridoidea (White, Cheney and Key, 1963). Indeed there are only three other clear cases of parthenogenesis in the whole of the true Orthoptera—or Saltatoria, and only one of these—*Saga pedo*—has been studied cytogenetically. Unlike this tettigoniid parthenogen which is tetraploid, *Moraba virgo* is diploid ($2n = 15$) and its chromosome complement is believed to undergo a premeiotic doubling with subsequent meiotic synapsis restricted to the sister products of this process (White, 1966). A diploid male complement of 17 chromosomes is very common among the Morabinae; such complements include two pairs of large metacentrics designated as AB and CD, six pairs of small acrocentrics (1-6 in number) and a medium sized X, but reductions in chromosome number have been produced by centric fusion on several occasions. There are several features peculiar to that of *M. virgo*: (1) there is a single small metacentric chromosome (termed " M_2 ") and two unmatched small acrocentrics; (2) two of the larger elements are also difficult to match—one is a submetacentric while the other is a metacentric—and they have been termed " CD standard " and " CD inverted " since they are thought to be homologous with the submetacentric CD chromosome pair that are a relatively constant component in other morabine species; (3) these same two large " CD " chromosomes show marked " heterozygosity " for late-labelling segments when tritiated thymidine is incorporated towards the end of the DNA-S-phase, as too do the two largest metacentrics termed " AB " (White and Webb, 1968). In particular, the short arm of the " CD standard " is very clearly late labelling; the one arm of one " AB " chromosome and parts of the " CD inverted " are also late labelling, and often there is moderate late labelling on the one arm of the " M_2 " and one or two of the small acrocentrics as well. This terminology has recently been

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modified (Webb and White, 1974) so that the " X " and " CD " pairs have been interchanged, and the " M_2 " renamed the X_2 in line with its presumed origin from an unknown X_1X_2Y progenitor species. However, the original terminology will be used herein to avoid complication.

These observations have led to the postulate that the complement of *Moraba virgo* is undergoing a process of " haploidisation " wherein the late replicating regions are being inactivated so that their functions are confined to the euchromatic regions of their homologues. The small metacentric " M_2 " is considered an essentially monosomic element so that it too is " haploidised ", while the " CD inverted " is also interpreted as providing further evidence for the trend to heterozygosity in this singular parthenogen (White and Webb, 1968; White, 1970; White, Webb and Cheney, 1973; Webb and White, 1974). If such a process is really occurring, then it is unique in the eukaryotes, and before accepting this interpretation simpler possibilities should be fully explored.

A common origin for parthenogenetic forms is hybridisation. Quite convincing examples exist in the simuliid *Cnephia mutata* (Basrur and Rothfels, 1959), in the lizard genera *Cnemidophorus* and *Lacerta* (*e.g.* Darevsky and Danielyan, 1968; Lowe *et al.*, 1970), in the fish *Poecilia formosa* (Abramoff *et al.*, 1968) and *Poeciliopsis* (Schultz, 1969), and in the Salamander *Ambystoma* "*jeffersonianum*" complex (Uzzell and Goldblatt, 1967). A similar genesis by hybridisation for *Moraba virgo* has been dismissed because of apparent homozygosity for the " X " chromosome fusion and because no species related to *M. virgo* appeared to offer a suitable ancestry (White, 1970, 1973).

Moraba virgo is found in the East of Australia in sandy areas of Western New South Wales and North Eastern Victoria on Acacia shrubs. Its known near relatives, *P125*, *P151*, *P152*, *P169*, *P188* and *P196*, all occur in Western Australia (White and Webb, 1968; White, Webb and Cheney, 1973) and two years ago *M. virgo* itself was also found in this region (White, 1974). Over 1000 miles separates these eastern and western distributions and it is probable that they were divided by the climatic changes of the Pleistocene and the Recent " great arid " period (*c.* 5000 B.P.). It therefore seems likely that *M. virgo* arose from species similar to its near relatives in Western Australia and extended its range beyond that of these bisexual forms, a not uncommon occurrence with parthenogens. Of the bisexual relatives the species *P196* and *P151* are most similar to *M. virgo*, and the possession of an X_1X_2Y sex mechanism in *P196* with a small metacentric X_2 similar in size to the " M_2 " of *M. virgo* has led to the current hypothesis that both *M. virgo* and *P196* arose from an unknown or extinct X_1X_2Y species by further chromosome rearrangements (White, Webb and Cheney, 1973).

Now morabine grasshoppers are wingless and appear to have relatively little effective premating isolation, since the various members of the *viatica* and *cultrata* groups can be successfully hybridised both in the laboratory and in the wild (White and Cheney, 1966; White, Blackith, Blackith and Cheney, 1967; White, Key, André and Cheney, 1969). Consequently one might imagine that sporadic hybridisation could have occurred between the bisexual relatives of *M. virgo*, and that on one such occasion the hybrid underwent premeiotic doubling to produce a form stabilised by thelytoky. The hypothesis that follows shows that the peculiar karyotype and late replication patterns can be simply explained if *M. virgo* is assumed to have

originated in such a manner following hybridisation between two forms similar to the extant West Australian relatives, *P196* and *P169*.

2. The hypothesis

At the top of fig. 1 is the basic morabine karyotype which is found in two members of the *virgo* group, *P151* and *P152*; this basic lettering follows that of White and Webb (1968). From this karyotype one can derive those of *P196* and *P169*. This involves three changes (*a*) dissociation of AB, (*b*) XA fusion and (*c*) B4 fusion. At least two modes of deriving *P196* can be envisaged, the first one of these, and that of *P169*, are essentially those presented previously (White and Webb, 1968; White, Webb and Cheney, 1973), the only change in the scheme offered here is that the small chromosomes have been renumbered to accommodate the known late labelling patterns in *M. virgo*. One cannot exclude other combinations of small chromosomes at present, since the 4, 5 and 6 are similar in size. The second scheme for deriving *P196* leads to the production of a small metacentric with a different composition. Both *B196* pathways involve two centric fusions, one tandem fusion and one peri-centric inversion. There is independent evidence that structural changes of the required kind have occurred frequently in the evolution of the Morabinae (White, 1973, p. 372 *et seq.*).

When the haploid complements of *P196* and *P169* so derived are combined the resulting karyotype is remarkably like that of *Moraba virgo* in size and composition, though it now requires a renumbering of the chromosomes, and in what follows the previous numbering will be in parentheses. Thus one " AB " is now in fact an XA from *P169*; the " X " pair are now the CD pair—unchanged and homomorphic; the " CD inverted " becomes the X1 from *P196* while the " CD standard " becomes the B4 from *P169*; the " M_2 " appears to be the X_2 from *P196* as White, Webb and Cheney (1973) surmised (but without recourse to their unknown intermediate ancestors), whose precise composition depends on the mode of origin adopted for *P196*. With the exception of 2 and 3 the remainder of the small acrocentrics are no longer in pairs. On the hypothesis offered here the suggestion that the pair called " CD " may be the " X " (White, 1973; Webb and White, 1974) is only half true, the X elements are in the AX and the X1 (or X56) chromosomes.

The anomalies mentioned previously are now readily accommodated by this model. Firstly, the " M_2 " chromosome is the X_2 (56 or linv) of *P196*. Secondly, the unusual heteromorphism in the " CD pair " stems from the fact that these two chromosomes are in no sense homologous. Thirdly, the general labelling pattern in morabines involves a late labelling megameric pair, with a moderately late X and other small pair (Webb, 1973). Thus the " heterozygosity " for late labelling in the " AB " pair is a result of the late replication of the X-arm of the *P169* neoX (XA), and only one member is actually an AB chromosome. The " heterozygosity " between the " CD inverted ", and " CD standard " has a two-fold origin, the X-arm of the X1 from *P196* shows some late labelling, while the 4-arm of the B4 from *P169* is very markedly late replicating and probably represents the megameric chromosome of the basic morabine complement (herein chromosome 4). In some figures of autoradiographs the one arm of the " M_2 " is late

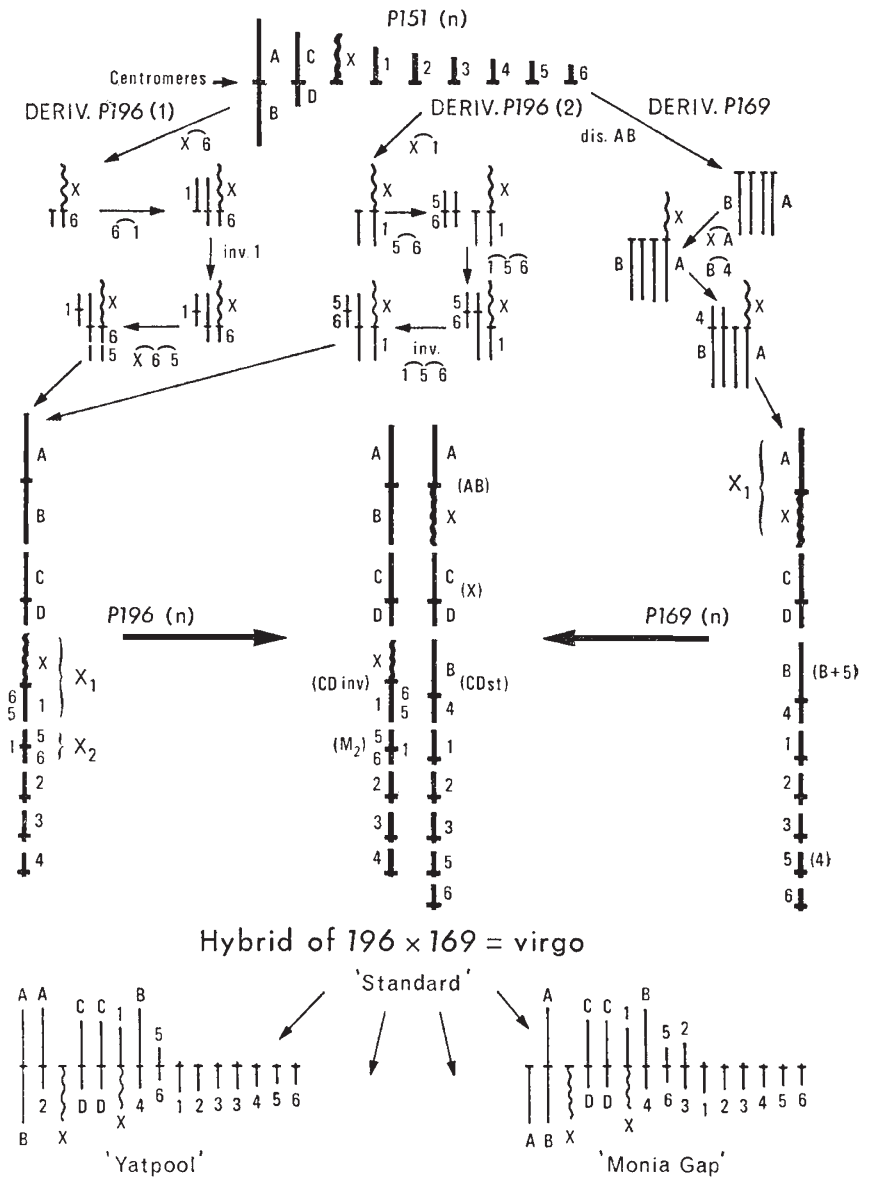

FIG. 1.—The proposed evolutionary pathway of the karyotype of *Moraba virgo* Key. At the top is *P151* from which *P196* and *P169* are derived through the partial karyotypes of hypothetical intermediates. The central " standard " *virgo* complement is derived from the flanking *P196* and *P169* by hybridisation, with further evolution to the variant *virgo* karyotypes at the bottom. The chromosomes are labelled following their derivation from the basic karyotype; labels in brackets are after White and Webb (1968) for reference. All the chromosome lengths are drawn to scale from the measurements of White and Webb (1968), White, Webb and Cheney (1973). Chromosomes paired in the *virgo* complement are shown the same length because the data have been recorded in this manner. Chromosome lengths are also known to vary relatively with stage and sex. The small acrocentrics are of similar size and therefore not absolutely characterised by length; chromosome 4 is chosen as the megameric and chromosome 6 as the other moderately late labelling acrocentric.

labelling (White and Webb, 1968, figs. 11, 12, 13 and 15) and this could be due to the fact that a second small acrocentric (herein chromosome 6) is also moderately late replicating. An alternative possibility is that the X_2 became somewhat heterochromatinised following its derivation as a neo-sex chromosome, although this process is more characteristic of neoY chromosomes in Orthoptera.

Another point that emerges from this biparental derivation of the *M. virgo* complement is that the late replicating regions of the *P169* chromosomes (*i.e.* the X-arm of the AX, and the 4-arm of B4) are more clearly late labelled than the corresponding parts of the *P196* chromosomes (*i.e.* the X-arm of X1, and the small acrocentric 4). A similar disparity between the two CD chromosomes (" X " in figs. 11-16 of White and Webb, 1968) where one appears more heavily labelled is not statistically distinguishable, however (Webb, 1973). Furthermore, the A-arm of the AX from *P169* is more heavily labelled than the equivalent A-arm in the AB from *P196*, and these observations suggest that the chromosomes contributed by *P169* are in general more late replicating than those contributed by *P196*. An equivalent situation is known in somatic cell hybrids where each genome retains its own S-phase duration—so that the chromosomes with the longer S-phase are the last to finish replication in the hybrid cell (Marshall-Graves, 1972). If this suggested pattern of labelling is confirmed in *M. virgo*, then one might expect *P169* to have a longer S-phase than *P196*. In this context it is interesting to note that *P169* is reported to have a higher nuclear DNA content (13·58 pg) than *M. virgo* (12·48 pg) (White and Webb, 1968), and it is possible that *P196* will have an even lower DNA value when it is determined (by subtraction this should be in the order of 11·38 pg). The higher DNA content of the *P169* genome may thus explain its apparent later replication.

In addition to the " Standard " *M. virgo* karyotype found in most localities, four others have been reported in isolated populations from Yatpool, Monia Gap, Connor's Tank and Hayes Hill. These are easily derived from the " Standard " *virgo* karyotype, and the two best recorded ones are shown under the proposed new classification in fig. 1. Of the other two, " Connor's Tank " is a report of one individual with essentially a " Standard " complement except for a centric fusion between two dissimilar small acrocentrics; " Hayes Hill " is very comparable with " Connor's Tank ", but the two fused small acrocentrics appear not to be the same ones. The late labelling patterns for " Yatpool " and " Monia Gap " (White and Webb, 1968, figs. 14-16) also fit neatly with the proposed hybrid derivation of *M. virgo*. These clear similarities with the " Standard " *virgo* complement, and the single individual at " Connor's Tank " which is in Eastern Australia well separate from the extant bisexual relatives in the west, argue for these four non-standard complements being the result of karyotypic changes subsequent to the establishment of *M. virgo* and derived from it. Alternatively, it is possible that some of these karyotypes also involved hybridisation between other bisexual forms. An examination of the known bisexual karyotypes shows this to be most unlikely, for although *P196* × *P125* has a distinct similarity to the " Monia Gap " *virgo*, the X chromosome in *P125* is metacentric while the free X in *virgo* is acrocentric.

There may have been other small rearrangements, duplications and losses, in the karyotypes of *P196*, *P169*, *P151* and *virgo* since the origin of the parthenogen that remain cryptic for the present.

3. Some predictions

The above hypothesis for the origin of *M. virgo* by hybridisation makes several clear predictions which are testable by detailed autoradiographic analyses coupled with careful measurement of the various chromosomes. (1) The B4 should be larger than the X1 (" CD " pair in previous terminology), the AB should be larger than the AX (" AB " pair previously), and the " Yatpool " free X should be equal to the X-arm of the X1 fusion which is currently confounded with the AB. (2) If *P169* contains the B4 of *virgo*—where 4 is defined as the late labelling megameric chromosome—then a large submetacentric should show similar short arm labelling in this species. (3) If *P125* did donate the second small metacentric (" M_1 ") to " Monia Gap " *virgo* (which seems unlikely) then these two chromosomes should be similarly labelled—or unlabelled—in *P125* in *M. virgo*. The extensive labelling studies advocated will allow a check on the possible difference between the DNA-S-phase in the *P169* and *P196* genomes, and this may be compared with the DNA content and S-phase values determined directly from the two putative parent species.

Two other methods of analysis might yield crucial information in assessing the precise mode of origin of *M. virgo*. (1) Hybridisation: If hybrids are possible between *P196* and *P151* then one may be able to demonstrate which chromosomes compose the X_1 and X_2 of *P196* and hence *virgo*, especially if the chromosomes show a distinctive pattern of banding following treatment with Giemsa or Fluorochromes. Hybridisation between *P169* and *P196* clearly should be attempted. Any hybrids might be expected to have morphological similarities with *virgo*, unless *P169* has diverged considerably since the origin of *virgo*. The karyotype should resemble that of *virgo*. Any gametogenesis should be examined for signs of premeiotic doubling. Indeed, a whole range of crossing between members of the *virgo* group could be informative. White (1974) has recently succeeded in producing viable triploids by crossing *virgo* with *P169* and *P196*. Thus here is further superficial similarity between the *virgo* case and that of other parthenogens which show hybrid-polyploid evolutionary complexes. (2) Electrophoresis: The application of electrophoretic techniques, as used in *Ambystoma* and *Poecilia*, to *P169*, *M. virgo* and *P196* could demonstrate *virgo's* proposed hybrid origin. The search for new members of the *virgo* group must of course continue.

4. Conclusion

A simple hybridisation model has been proposed to account for the origin of the only all female grasshopper *Moraba virgo*. This has the virtue of explaining the major anomalies in the puzzling and peculiar karyotype of this parthenogen. The model is predictive and studies on chromosome length related to replication behaviour in *virgo* and its bisexual relatives, coupled with hybridisation studies, offer clear means of testing the hypotheses.

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5. References

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