Further Studies on the Cytology and Distribution of the Australian Parthenogenetic Grasshopper, *Moraba virgo*

by

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with 3 figures

To Prof. J. Seiler, pioneer investigator of the cytology of parthenogenesis, on his 80th birthday.

1. INTRODUCTION

In a recent paper (White, Cheney and Key 1963) the occurrence of an all-female species of grasshopper, *Moraba virgo* Key (*Orthoptera, Eumastacidae, Morabinae*) at three localities in New South Wales has been reported. The cytogenetic mechanism of parthenogenesis in this insect is quite peculiar: a supplementary chromosomal replication occurs in the oocyte before synopsis and is followed by a normal two-division meiosis, with as many bivalents as there are chromosomes in the somatic nuclei. The species is a complex heterozygote for various inversions, fusions and translocations of its chromosomes. Synopsis only occurs between sister

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chromosomes resulting from the supplementary replication (which are necessarily molecular copies of one another), so that no multivalents or structurally heterozygous bivalents are formed. The chromosomal heterozygosity of an individual is thus transmitted to all its progeny.

Several further collecting trips in November-December 1962, January-February 1964 and January 1966 have greatly extended our knowledge of the distribution and ecology of the species, and have provided material for some further cytological studies which will be reported here.

2. DISTRIBUTION

The 19 localities at which Moraba virgo has been collected are as follows:

1. 9 miles W. of Boundary Bend, Victoria
2. Nowingi, Victoria
3. 0.5 miles S. of Yatpool, Victoria
4. 15 miles S. of Pooncarie, N.S.W.
5. 7 miles E. of Pooncarie, N.S.W.
6. South Ita Sandhills, N.S.W.
7. 10 miles S. of Connor’s Tank, N.S.W.
8. 2 miles E. of Little Topar Tank, N.S.W.
9. 2 miles E. of Hazelvale, N.S.W.
10. 3 miles E. of Worungil Tank, N.S.W.
11. 1 mile W. of Caltagena Tank, N.S.W.
12. 1 mile SW of Coonavitrtra Tank, N.S.W.
13. 4 miles W. of Cobar, N.S.W.
14. 3 miles SW of Shuttleton, N.S.W.
15. 3 miles E. of Roto, N.S.W.
16. 7 miles SE of Mount Hope, N.S.W.
17. 10 miles W. of Mt. Boorithumble, N.S.W.
18. 5 miles WNW of Euabalong West, N.S.W.
19. 9 miles WNW of Monia Gap, N.S.W.

These collecting stations are shown in figure 1, on which the circular symbols are located at the exact point where each collection was made, but are labelled in shortened form, i.e. the locality “9 miles WNW of Monia Gap” is simply labelled “Monia Gap”.
Fig. 1

Map of the region of southeastern Australia in which *Moraba virgo* occurs, showing the localities at which it has been collected. The black areas are lakes, many of which are normally dry. The approximate distribution limits of the two principal food plants, *Acacia wilhelminiana* and *Acacia loderi* are indicated.
Moraba virgo is a wingless species that inhabits shrubs and low trees. In the earlier paper it was only recorded from the rare shrub Acacia wilhelmiana F. Muell. We now know that it occurs on several other species of Acacia as well. At localities 1, 2, 4, 6, 15, 18 and 19 the insects were found exclusively on Acacia wilhelmiana, which was usually the only species of Acacia present. At localities 7, 8, 9, 10, 11 and 12 they were collected on A. loderi Maiden which is a small tree when mature. At localities 16 and 17 the foodplant was A. rigens Cunn. At locality 3 the main foodplant was undoubtedly A. wilhelmiana, but some individuals were swept from shrubs of A. sclerophylla Lindl. growing nearby. Finally, at locality 13 a large population exists on dense growth of an Acacia which varies greatly in leaf shape but all the individuals of which would, according to current taxonomic concepts, be included in A. aneura F. Muell. ("Mulga"). In general, it can be said that in the southern part of its range, M. virgo feeds on A. wilhelmiana, while the more northerly populations live on A. loderi, but that throughout its distribution it occasionally occurs on other species of Acacia. The area it inhabits is an arid one and its two main food plants are confined to light, alkaline sandy soils. A. wilhelmiana is not a common species, i.e. it occurs as isolated colonies usually covering a few hectares. It is a component of the "mallee" vegetation, while A. loderi occurs in the Casuarina-Heterodendron Association of Beadle (1948) and in Mulga scrub where it forms almost pure stands in certain areas. The occurrence of M. virgo on mulga (Acacia aneura) at the Cobar locality, almost certainly outside the ranges of both A. wilhelmiana and A. loderi, seems anomalous. Hundreds of trees of A. aneura further west, in the Broken Hill area, have been examined without M. virgo being found on them. But the mulga at the Cobar locality has much less leathery phyllodes than the form of the species which occurs west of the Darling River; it probably falls in the category of "Umbrella Mulga" of Everist (1949) and may well belong to a different species.

It seems likely that future collecting will reveal the presence of M. virgo in the state of South Australia, on A. wilhelmiana. But its range is probably almost limited to the areas (shown in figure 1) within which A. wilhelmiana and A. loderi occur. There is no reason to believe that it extends widely on mulga to the north or
west of the area shown in figure 1. On the west side of the Flinders Ranges *A. loderi* is replaced by a closely similar species, *A. sowdenii* Maiden, but extensive collecting on that species in several areas has failed to reveal any individuals of *M. virgo*.

The closest relatives of *M. virgo* are apparently two undescribed bisexual species of Morabine grasshoppers known only from arid regions of Western Australia, where they probably feed on *Acacia* spp. There is thus a gap of at least 1400 miles between the known range of *M. virgo* and those of its nearest relatives. None of the species of Morabine grasshoppers known from eastern Australia appear at all closely related to *M. virgo*.

### 3. CYTOLOGICAL OBSERVATIONS

In the previous paper two different karyotypes were recorded for *Moraba virgo*, one of which (the "Roto karyotype") was present at the Roto and Shuttleton localities, while the other was present at the Monia Gap locality. These karyotypes were studied in aceto-orcein squashes of ovaries from individuals injected with colcemid (CIBA) the day before. The chromosomes in the ovarian follicle cells are generally well spread by this method.

Later observations show that the "Roto karyotype" occurs at all the collecting localities except the ones near Monia Gap and Yatpool. At all these except locality 7 (10 miles S. of Connor's Tank) it seems, on the evidence available, to be the only karyotype present. We may therefore appropriately re-name it the Standard karyotype. Other, variant, karyotypes occur at the Monia Gap and Yatpool localities (where they seem to be found in all members of the population). Finally, at the Connor's Tank locality a single individual was found (out of 5 examined) which showed a third variant karyotype.

The *Standard* karyotype consists of fifteen chromosomes. Our nomenclature for these is in conformity with the one we have used for other species of Morabine grasshoppers (White 1956, 1966; White, Carson and Cheney 1964). There is a large pair of "AB" chromosomes with almost exactly equal limbs, a pair of "CD" chromosomes which are distinctly shorter, a pair which are somewhat shorter than the CD's and which we identify as the X-chromo-
somes and nine smaller elements. The CD pair is invariably heterozygous for a rearrangement that appears to be a pericentric inversion. One of the CD chromosomes is a very unequal-armed metacentric, while in the case of the other the two limbs are almost exactly equal in length. Since a very large proportion of the species of Morabine grasshoppers (including the two believed to be taxonomically most closely related to \textit{virgo}) have an unequal-armed CD, we have called the unequal armed CD of \textit{virgo} the "Standard" sequence, the equal-armed CD being referred to as having the "Inverted" sequence. Clearly, in this case, the Standard sequence is the original one, the equal-armed condition being derivative. The nine small chromosomes consist of eight acrocentrics, which apparently form 4 pairs of homologues, and a little unpaired metacentric ("m$_2$ ").

We give in Table 1, the lengths of the chromosome arms in the Standard karyotype, expressed as percentages of the total haploid chromosome length (strictly speaking, as percentages of half the diploid length, since we can hardly speak of a haploid complement in view of the presence of the m$_2$).

![Diagram](image_url)
The Monia Gap karyotype was fully described in the earlier paper (White, Cheney and Key 1963): it differs from the Standard one in that one of the AB chromosomes is dissociated into two acrocentrics and two of the small chromosomes (probably a member of the no. 2 pair and one of the no. 3 pair) are fused to give a meta-centric which we have called m\(_1\).

At the Yatpool locality all the individuals that have been examined (about 8 altogether) were heterozygous for a translocation between one of the AB chromosomes and one of the smaller elements (no. 2 or no. 3). This has produced two new chromosomes: an acrocentric which we have called A' and an unequal-armed metacentric which we have called B' but which cannot be distinguished from the Standard CD. The Yatpool karyotype has 8 small chromosomes (including the unique little metacentric m\(_2\)) instead of the usual 9.

In one of five individuals from the Connor's Tank locality it was found that 2n = 14. there being a centric fusion between one of the largest of the small chromosomes (no. 1 or 2) and one of the smallest. There is hence an unequal-armed metacentric (m\(_3\) in figure 3d) in addition to the little m\(_2\) chromosome. The other four individuals from the same locality showed the Standard karyotype. It must remain uncertain for the present whether this "Connor's Tank karyotype" occurred in a single individual or is more widely spread. The origin of the Connor's Tank karyotype clearly involved the loss

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* These figures are the means of three cells photographed and measured. The apparent difference in overall length between the Standard and Inverted CD chromosomes is probably not significant.
of some minute proximal regions from two chromosomes. The Yatpool karyotype, on the other hand, must have arisen by a simple mutual translocation, without any loss of material. And the most plausible origin for the Monia Gap karyotype would be a 3-break rearrangement, without any loss of material, producing a simultaneous dissociation and fusion.

4. DISCUSSION

*Moraba virgo* must be assumed to have been derived from a bisexual species with $2n^\varphi = 18$, the "primitive" Morabine...
karyotype which is, incidentally, present in the two Western Australian species believed to be most closely related to it. We therefore have to explain how the chromosome number has been reduced from 18 to 15 elements in the phylogeny of *M. virgo*.

The two Western Australian relatives of *M. virgo* are both XO(♂) species with 2n♂ = 17 (and hence 2n♀ = 18). Their karyotypes, which are virtually indistinguishable, comprise a pair of AB chromosomes, a pair of CD chromosomes, an X chromosome and six pairs of small acrocentric autosomes. Only one individual of each has been examined cytologically, but no structural heterozygosity was encountered.

It was suggested in the earlier paper that the little m₂ chromosome might have arisen by a fusion between two small non-homologous autosomes, and that the unfused homologues of its two arms might have suffered deletion from the karyotype. This suggestion, which would formally account for the reduction of the chromosome number from 18 to 15, seems rather unlikely on general grounds and a study of the two Western Australian species at once suggests another possibility.

The X chromosome of *Moraba virgo* is an unequal-armed metacentric whose length is about 18% of the total haploid chromosome length. The two Western Australian species both have rather short acrocentric X-chromosomes whose length probably does not exceed 10% of the total chromosome length. This at once suggests that the X-chromosome of *M. virgo* is a fusion chromosome, the short limb of which is of autosomal origin. If so, the immediate ancestor of *virgo* would have been a neo-XY species or population (probably now extinct) with 2n♂♀ = 16. Another possibility would seem to be that the "missing" pair of small autosomes has somehow become incorporated in the CD pair. This seems unlikely because (1) the CD is almost always a metacentric in Morabine grasshoppers and therefore unlikely to undergo centric fusion and (2) there seems to be no large scale discrepancy between the relative length of the CD in *virgo* and its relatives, such as exists in the case of the X. Since *virgo* is homozygous for the postulated X-autosome fusion this presumably arose in "bisexual times", before the establishment of thelytoky as the normal mode of reproduction.

The inversion in the CD pair could, theoretically, have established itself before or after the origin of thelytoky. The fact that
it is present in every individual of _M. virgo_ is _prima facie_ evidence for it having arisen before the thelytokous mode of reproduction. On the other hand it is possible that it arose later and that the lineage homozygous for the original sequence has become extinct.

There are two possibilities with regard to the origin of the little m₂ chromosome. On the one hand, it may have arisen by fusion between two non-homologous chromosomes. If so, it would be essentially homologous to the two smallest members of the Standard karyotype, which would not constitute a pair, in spite of the fact that they appear indistinguishable in length. On the other hand, it may have arisen by a fusion between the two members of a pair of homologues.

The two limbs of the m₂ are significantly different in length [ratio about 0.55: 0.45]. Their total length is quite significantly less than the sum of the lengths of the two smallest elements of the Standard karyotype. The latter fact seems to argue against the first hypothesis and in favour of a fusion between two homologous chromosomes; the first hypothesis would involve deletion of a significant proximal segment from both the chromosomes that underwent fusion, which seems unlikely. The second hypothesis would also require deletion of some genetic material, but this could be mainly from one of the fusing elements.

The little m₂ chromosome could not have been inherited in a regular manner in "bisexual times". This statement is true, regardless of whether it arose by fusion between non-homologous or homologous chromosomes. In the former case it is fairly unlikely that its two limbs would each have formed a chiasma with complete regularity; and there is the further problem of the regularity of orientation of such a small trivalent.

There are hence two alternatives: (1) that the m₂ chromosome has arisen since the origin of the thelytokous mode of reproduction, (2) that it did, in fact, exist in bisexual times, but imposed a considerable segregational load on the population (in the form of inviable aneuploid individuals).

In any case, it seems likely that the peculiar type of thelytokous reproduction found in _M. virgo_ arose by a mutation which gave rise to the supplementary premeiotic chromosomal replication. The mutation may have proved adaptive if it arose in a population which was characterized by heterotic polymorphism, i.e. one carrying
a considerable genetic load, in the form of adaptively inferior homozygotes. By abolishing segregation the mutation would have led to an immediate shedding of the genetic load. The fact that long-term adaptability would have been sacrificed, to a considerable extent, would have been of less immediate consequence. Whether the postulated genetic load was mainly due to the inversion system in the CD chromosome, to the little m² element, or to genic heterozygosity not associated with any chromosomal rearrangements, cannot be determined at present.

In a species with the type of chromosome cycle found in *Moraba virgo*, various types of chromosomal rearrangements that would stand little or no chance of evolutionary success in a bisexual species might be expected to establish themselves. There should be only two "mechanical" barriers to the establishment of chromosomal rearrangements in *M. virgo*. In the first place chromosomes incapable of passing regularly through the somatic mitotic cycle (i.e. acentrics, dicentrics and rings) will be eliminated. And, in the second place, every "rearranged" chromosome has to be capable of regularly forming at least one chiasma at meiosis. These restrictions should not affect inversions, whether paracentric or pericentric; and many types of translocations should, theoretically, be capable of establishing themselves in the karyotype.

There is now plenty of evidence for the occurrence of chromosomal rearrangements at a fairly high frequency in grasshopper populations (as distinct from their successful establishment in the species). A number of rearrangements (mostly translocations) have been described in single individuals of *Keyacris* (formerly *Moraba*) *scurra* (White 1956, 1961a, 1963) and *Trimerotropis gracilis* (White 1961b). They have also been described in *Chorthippus* spp. (Coleman 1947, Lewis and John 1963, John and Hewitt 1963), and in *Gesonula punctifrons* (Sarkar 1955). All these rearrangements were presumably unique ones, present in the germ line of a single individual and not encountered again in the population. It has been estimated (White 1964) that in natural populations of *Keyacris scurra* about one individual in 750 carries a viable, cytologically visible, newly-arisen rearrangement. There seems no reason to suppose that the frequency of such rearrangements would be any lower in *M. virgo*.

Thus, even on the conservative estimate that there may be 10,000 individuals of *M. virgo* per generation and that the species
has been in existence for 1000 years (i.e. generations), well over 10,000 viable and visible rearrangements would have occurred in
the germ line. It is far more probable that the species is of much
greater antiquity and that its population size may have been
considerably over 10,000, at least in the past. If so, the number of
rearrangements that have occurred in *M. virgo* since it adopted its
thelytokous mode of reproduction may well have been \(10^5\), \(10^6\) or
even higher. Out of all these, we have only been able to find 3 or
4 (if we count the fusion and the dissociation in the Monia Gap
karyotype as "separate" rearrangements). It is, of course,
probable that further collecting would reveal additional colonies
like the Yatpool and Monia Gap ones, with new and unique arrange-
ments. But even so, the conclusion is inescapable that only a
minute fraction of the rearrangements that have occurred have
survived the sieve of natural selection.

It would therefore seem clear that deleterious position effects
must account for the elimination from the population of the over-
whelming majority of spontaneous rearrangements. It certainly
seems significant that all the chromosome breaks responsible for
the rearrangements in the Monia Gap, Yatpool and Connor's Tank
karyotypes seem to be close to the centromeres. They consequently
gave rise to fusions, dissociations or translocations of whole arms.
The absence of pericentric inversions (except for the one in the CD,
which presumably arose before the origin of thelytoky in *M. virgo*)
is perhaps the most unexpected feature of the cytogenetics of the
species, and the one which points most strongly to the near-
universality of deleterious position effects of rearrangements.

No male individual of *M. virgo* has been encountered. The
only way in which such an individual could be expected to arise
would be as a result of the loss, by non-disjunction, of one X from
the karyotype. In view of the probability that the X of *M. virgo*
is a fusion-X, it is quite possible that XO individuals would be
inviable at an early stage of development.

Seiler (1961) has been concerned with the origin of thelytokous
reproduction in species of Lepidoptera inhabiting the mountainous
regions of central Europe, heavily glaciated in recent times. The
area of the world's surface occupied by *Moraba virgo* is widely
different — a level arid plain covered by a semi-desert sclerophyllous
vegetation composed of a great variety of shrubs and small trees.
Yet climatic changes must have occurred in this region also during the glacial epoch. It seems likely that even 10,000 years ago the area suitable for occupation of \textit{M. virgo} (or its ancestors) would have been considerably to the north or northwest of its existing position. It is thus not improbable that the present gap between the ranges of \textit{M. virgo} and its western relatives is due to a displacement south-eastward of the mallee and Casuarina-Heterodendron Association from a position much nearer Central Australia.

By comparison with many bisexual thamnophilous species of Morabine grasshoppers, \textit{M. virgo} lives on a limited range of food-plants, since there is no evidence that it normally feeds on any of the species of \textit{Dodonaea, Melaleuca} or \textit{Olearia} which grow in the localities where it occurs and which furnish food for other Morabine species. The limited diet of \textit{M. virgo} may be regarded as an indication of restricted adaptability due to lack of genetic recombination.

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SUMMARY

The parthenogenetic eumastacid grasshopper \textit{Moraba virgo} occurs throughout a wide area of southeastern Australia, where it feeds on several species of \textit{Acacia}. No males have been encountered and reproduction is exclusively thelytokous. The Standard karyotype shows $2n = 15$, with a single small metacentric chromosome. The second largest chromosome pair is always heterozygous for a pericentric inversion. Several karyotypes with additional fusions, dissociations and translocations occur at single localities.
The chromosome number in the oocyte is doubled before the beginning of the maturation divisions and synopsis is restricted to sister chromosomes so that 15 structurally homozygous bivalents are formed. There are two meiotic divisions in the egg. This mechanism ensures the perpetuation of the heterozygous constitution. Comparison with related species suggests that \textit{M. virgo} has been derived from a species with $2n\varphi = 18$, following a fusion between the X and a small autosome and a second fusion between autosomes which gave rise to the small metacentric element.

\begin{flushright}
\textbf{CONCLUSIONS}
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La sauterelle parthénogénétique \textit{Moraba virgo (Eumastacidae)} a été trouvée dans une région étendue du sud-est de l'Australie, où elle vit sur diverses espèces d'\textit{Acacia}. Le mâle est inconnu et la
reproduction strictement thélytoque. Le caryotype “Standard” a 2n = 15, le nombre impair étant dû à la présence d’un petit chromosome métacentrique. La seconde paire de chromosomes est toujours hétérozygote pour une inversion péricentrique. Plusieurs caryotypes avec d’autres fusions, fissions et translocations chromosomiques ont été trouvés dans des localités isolées. Le nombre chromosomique est doublé avant le commencement de la maturité et la synapse a lieu uniquement entre chromosomes-sœurs; 15 bivalents structurellement homozygotes sont formés par l’appariement des 30 chromosomes. Il y a deux divisions de maturité dans l’oocyte. Ce mécanisme réalise la perpétuation de la constitution hétérozygote. Une comparaison avec des espèces voisines suggère que M. virgo dérive d’une souche avec 2n♂ = 18, par une fusion du chromosome X avec un petit autosome et une deuxième fusion qui a donné lieu au petit chromosome métacentrique.

REFERENCES


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