# Laboratory studies on hybridization among Namib Desert Tenebrionids (Coleoptera: Tenebrionidae)

by

D.C. Osberg

S.A. Hanrahan

Department of Zoology
University of the Witwatersrand
1 Jan Smuts Avenue
Johannesburg
2001
Republic of South Africa

and

M.K. Seely

Desert Ecological Research Unit
P.O. Box 953
Walvis Bay
9190
South West Africa/Namibia

CONTENTS

ABSTRACT

The genetic possibility of putative intergeneric hybridization between two sympatric Namib tenebrionids is described. Crosses and reverse crosses were carried out between the putative parent species, as well as between two other related taxa. Egg production and hatching success were recorded for intergenerically, intersubspecifically and intraspecifically mated females as well as for females that were isolated from males and these data were compared. Obvious differences in the morphology of hybrid and nonhybrid larvae are described.

## 1  INTRODUCTION

The natural occurrence of animal hybrids is rare and therefore any finding of such deserves mention in the literature. Often the claim that an animal is a hybrid has no more solid foundation than that a possibly mutant type happens to have characters that are a mixture of those of two different species (Gray, 1972). Two morphologically intermediate adult tenebrionid individuals have been collected in the dry Kuiseb River bed near the Namib Research Institute at Gobabeb, South West Africa/Namibia. One individual, a male, has been described (Hamilton and Penrith, 1977) and it has been suggested, on morphological grounds, that this individual may be a hybrid derived from the two sympatric species, *Onymacris rugatipennis rugatipennis* (Haag) and *Physadesmia globosa* (Haag). Other suggestive evidence for possible hybridization between these two species includes the sympatric occurrence of large numbers of both species along the Kuiseb River bed (Fig. 1). The high degree of overlap in use of time and space by both species, the apparently ambivalent courtship patterns of both putative parent species and the "intermediate" choice of females of both species by the putative hybrid male in courtship (Hamilton and Penrith, 1977; Hamilton *et al* 1976). In this paper, we have tested the genetic possibility of hybridization between these two species through laboratory breeding experiments.

Also occurring sympatrically with *O. r. rugatipennis* and *P. globosa* in the river bed, is *Physosterna cribripes* (Haag) (Plate 1, Fig. 1). *P. globosa* is broadly morphologically similar to *P. cribripes* and was initially placed
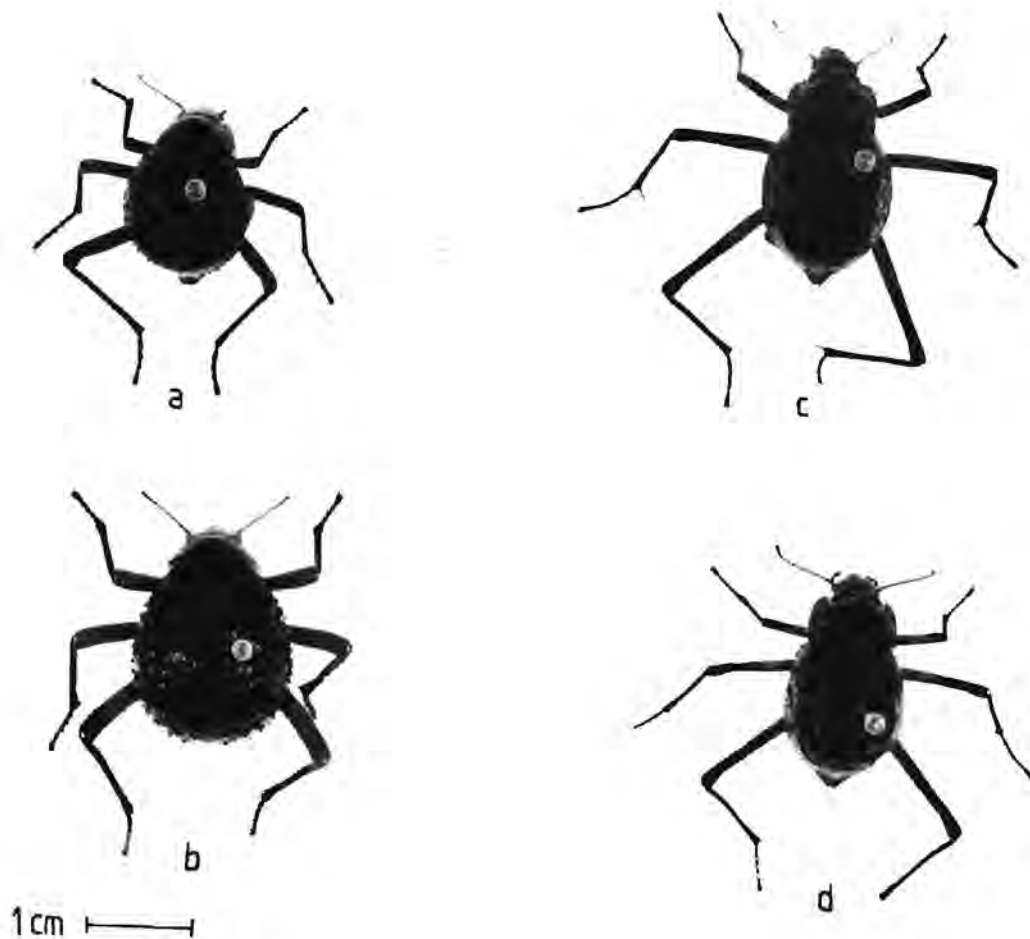
PLATE 1: a) *Physadesmia globosa*, b) *Physosterna cribripes* c) *Onymacris rugatipennis rugatipennis* d) *Onymacris rugatipennis albotessellata*.

in the same genus. However it has since been described as being, in many ways, intermediate between *Onymacris* and *Physosterna* and has recently been placed in a separate genus (Penrith, 1979). Although *O. r. rugatipennis* and *P. globosa* are, on morphological grounds and for other reasons, the most likely parent species, we included crosses between *O. r. rugatipennis* and *P. cribripes* for comparative purposes.

*O. r. rugatipennis* and *O. r. albotessellata* Schulze, were also mated in the laboratory. These two groups have been classified as subspecies because of their identical morphology in the adult phase (Penrith, 1975). They are, however, easy to distinguish in the field as *O. r. albotessellata* always exhibits a prolific "waxy bloom" , which *O. r. rugatipennis* cannot be induced to produce, even under laboratory conditions (Plate 1). Their ranges are contiguous, even overlapping slightly in the Kuiseb River bed (Fig. 1) but there is no known evidence of introgression between these two groups. The object of crossing them in the laboratory was to test whether they were fully interfertile.

## 2 MATERIALS AND METHODS

Although it is desirable to use virgin females in crossing experiments, only three virgin female *O. r. rugatipennis* individuals that had been reared in captivity were available at the start of this study. Desert tenebrionids are particularly difficult to rear in captivity as to date little is known about the conditions that are required for their survival in the immature stages of their life cycle. Because three was obviously an insufficient number of individuals for the purpose of this study, other individuals of each of the four taxa were collected from the field. Females that were to be used in crossing experiments were isolated from males and experiments were started after the females had produced only inviable eggs for a period of approximately ten weeks. Crossing experiments were carried out for five weeks.

Crosses and reverse crosses using field captured individuals were carried out between *O. r. rugatipennis* and each of the other taxonomic groups. In addition,

vegetated dunes

*O. r. albotessellata*

dune/river border

*O. r. rugatipennis*

river bed

*P. globosa*

*P. cribripes*

gravel plains

FIGURE 1: Diagram to represent the overlapping ranges of the four beetle populations.

some of the field captured females were isolated from all males, while others were used as controls and allowed to mate intraspecifically.

Each of the three virgin female *O. r. rugatipennis* individuals that had been reared in captivity was crossed with a male of one of the three other taxa and these crosses were run concurrently with the crosses using field-captured females.

The beetles were kept in plastic two litre containers, approximately 10 cm deep and provided with a shallow layer of Namib dune sand in which to oviposit. They were fed on oats and lettuce and kept at a temperature of approximately 30°C. On a weekly basis the eggs were sieved from the sand in each container and set aside to hatch. A record was kept of the total number of eggs laid by each group each week and the percentage of these that hatched.

An attempt was made to rear the larvae in 250 ml glass jars containing a mixture of Namib dune sand mixed with crushed oats. A small patch of sand at the bottom of each jar was kept moist to prevent the larvae from dehydrating. Three larvae in the first or second instar were introduced into each jar and after five months the widths of the head capsules of all surviving larvae were measured. Comparisons were made between the head width measurements of hybrid larvae and those of non-hybrid larvae. Mortality among larvae was unusually high and, although some distinct trends were shown statistically, the low numers of larvae remaining at this stage renders the interpretation of these statistical tests questionable.

Schulze (1962, 1964) has described the species specific characters of the ninth abdominal tergite of the larvae of these beetles. In order to investigate differences in morphology of hybrid and non-hybrid offspring, the ninth abdominal tergites of all larval offspring were examined under a dissecting microscope and photographed.

At the end of the experiment the spermathecal glands of five adult female individuals from each cross and each control were dissected out in saline solution and squash preparations were examined under a dissecting microscope.

## 3 RESULTS

### 3.1 Egg production

Females mated to conspecific males continued to lay eggs throughout the duration of the experiment. The hatching success of these eggs fluctuated widely from week to week, although the overall mean was high (Fig. 2, 5, 8, 11). Females isolated from males continued laying eggs throughout the experiment although they produced fewer eggs than control females (P< 0.05). Several weeks after isolation only in-

viable eggs were produced by isolated females (Fig. 4, 7, 10, 15).

Intergeneric crosses between field captured *O. r. rugatipennis* and *P. cribripes* individuals, resulted in a decrease in egg production compared with that of control females (P < 0.05) in whichever direction the cross was run. The number of eggs laid by these females was not significantly different from the number laid by isolated females (P > 0.1) and none of these eggs hatched (Fig. 3, 12). Attempts at mounting were infrequent compared with the mating behaviour of control individuals, and observed only in the *O. r. rugatipennis* female X *P. cribripes* male cross.

Intergeneric crosses between field captured *O. r. rugatipennis* and *P. globosa* individuals produced different results for each direction of the cross although mounting and pairing was frequently observed in both. There was no significant decrease in egg production by *O. r. rugatipennis* females mated to *P. globosa* males, compared with that of control females (P > 0.2), but a slight decrease in the hatching success of these eggs (P < 0.05) was observed (Fig. 13).
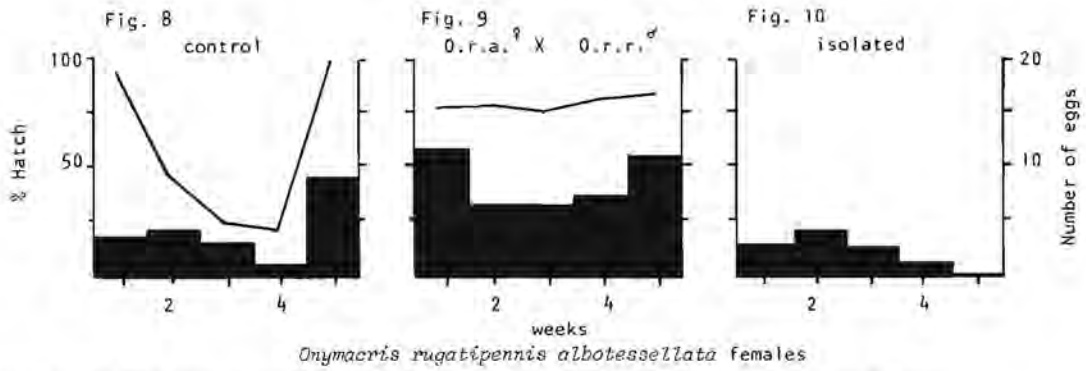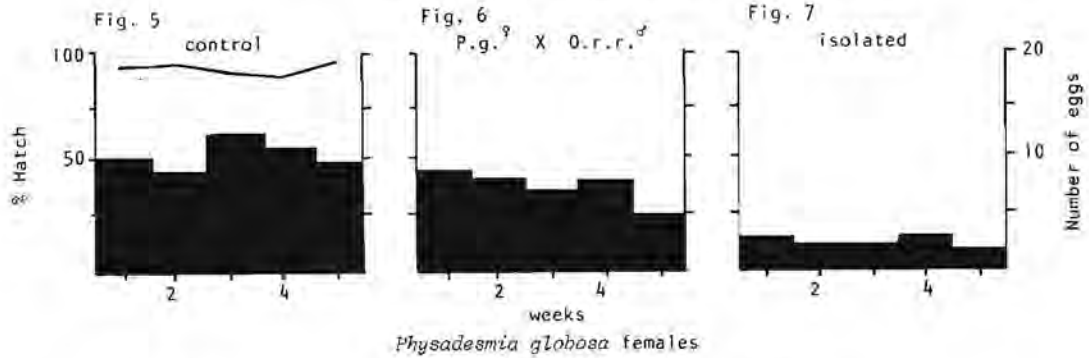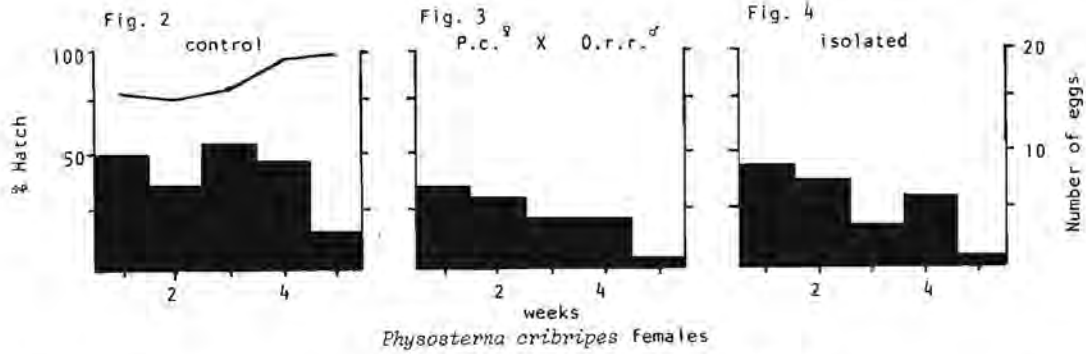
*P. globosa* females mated to *O. r. rugatipennis* males, on the other hand, showed a decrease in egg production compared with that of control females (P < 0.01), although egg production still remained at a level slightly higher than that of isolated females (P < 0.01). None of the eggs hatched (Fig. 6).

Mating between field captured *O. r. rugatipennis* and *O. r. albotessellata* individuals was frequently observed and there was no significant decrease in egg production in whichever direction the cross was run (P > 0.2). However, while the eggs laid by *O. r. rugatipennis* females mated to *O. r. albotessellata* males showed a slight decrease in hatch success (P < 0.05), the eggs laid by *O. r. albotessellata* females mated to *O. r. rugatipennis* males showed a slight increase (P < 0.05; Figs. 9, 14).
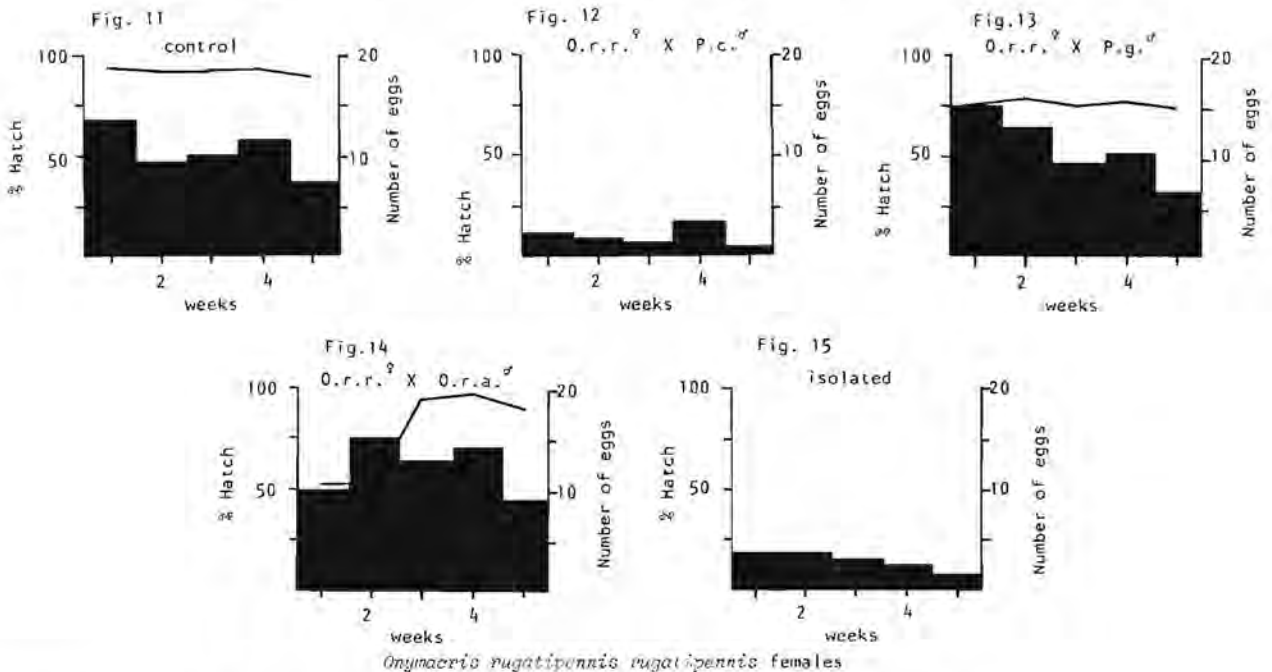
Hatching success of eggs from the three virgin females cross mated with different males was similar to hatching success of eggs from field-captured females. The cross between the *O. r. rugatipennis* virgin and *P. cribripes* male was completely unsuccessful. None of the eggs produced by this female hatched. The *O. r. rugatipennis* virgin that was crossed with a *P. globosa* male produced some viable eggs as did the virgin *O. r. rugatipennis* female that was crossed with an *O. r. albotessellata* male.

### 3.2 Larvae

Measurements of the widths of the head capsules of the surviving larvae, taken after five months growth, revealed some marked differences in size between larvae of different crosses. The larvae (n=3) resulting from the intergeneric cross between the *O. r. rugatipennis* female x *P. globosa* male were significant-

Fig. 2 — control

Fig. 3 — P.c. ♀ X O.r.r. ♂

Fig. 4 — isolated

*Physosterna cribripes* females

Fig. 5 — control

Fig. 6 — P.g. ♀ X O.r.r. ♂

Fig. 7 — isolated

*Physadesmia globosa* females

Fig. 8 — control

Fig. 9 — O.r.a. ♀ X O.r.r. ♂

Fig. 10 — isolated

*Onymacris rugatipennis albotessellata* females

FIGURES 2—10: Egg hatching success (—) and mean number of eggs produced per female per week (■).

Fig. 11 — control

Fig. 12 — O.r.r. ♀ X P.c. ♂

Fig. 13 — O.r.r. ♀ X P.g. ♂

Fig. 14 — O.r.r. ♀ X O.r.a. ♂

Fig. 15 — isolated

*Onymacris rugatipennis rugatipennis* females

FIGURES 11—15: Egg hatching success (—) and mean number of eggs produced per female per week (■).

ly smaller than larvae (n=4, p < 0.01) resulting from intersubspecific (*O. r. albotesselata* or *O.r. rugatipennis*) intraspecific matings which were, more or less, of equivalent length.

An examination of the ninth abdominal tergite, in the larvae derived from the *O. r. rugatipennis* female x *P. globosa* male cross, revealed it to be intermediate between both parental types (Plate 2). While the setal arrangement was more similar to that normally characteristic of *O. r. rugatipennis*, the apex of the ninth abdominal tergite, instead of being rounded as in *O. r. rugatipennis*, was pointed — a feature characteristic of *P. globosa* larvae.

Larvae are easily distinguishable from each other by the relative degree of pointedness of the apex of the ninth abdominal tergite and the relative length of the main setae on this tergite. Interbreeding between *O. r. rugatipennis* and *O. r. albotessellata* resulted in larvae that were intermediate between both types (Plate 3).



b) hybrid between (a) and (c), with pointed apex, short setae and anterior bare part two and a half times longer than apical setose part;
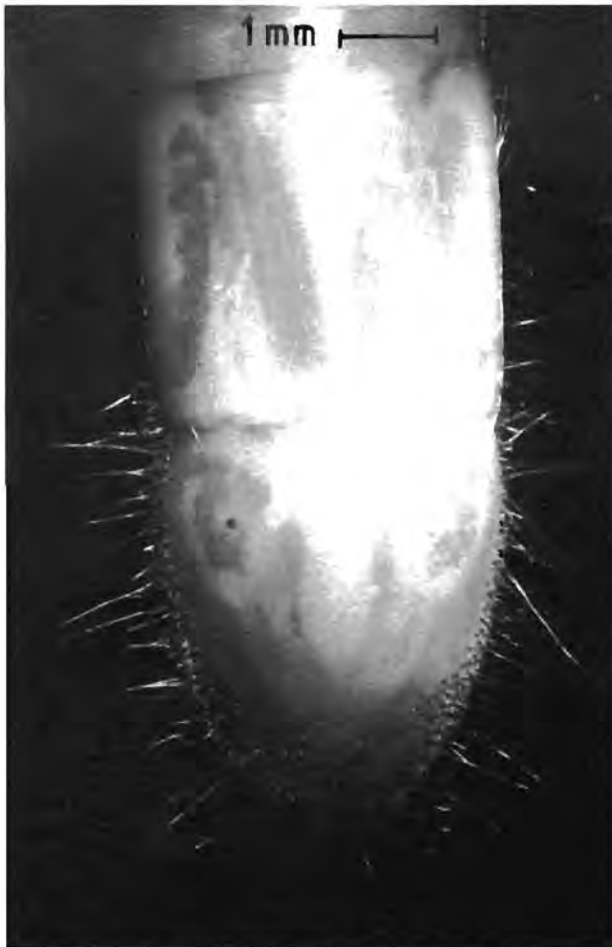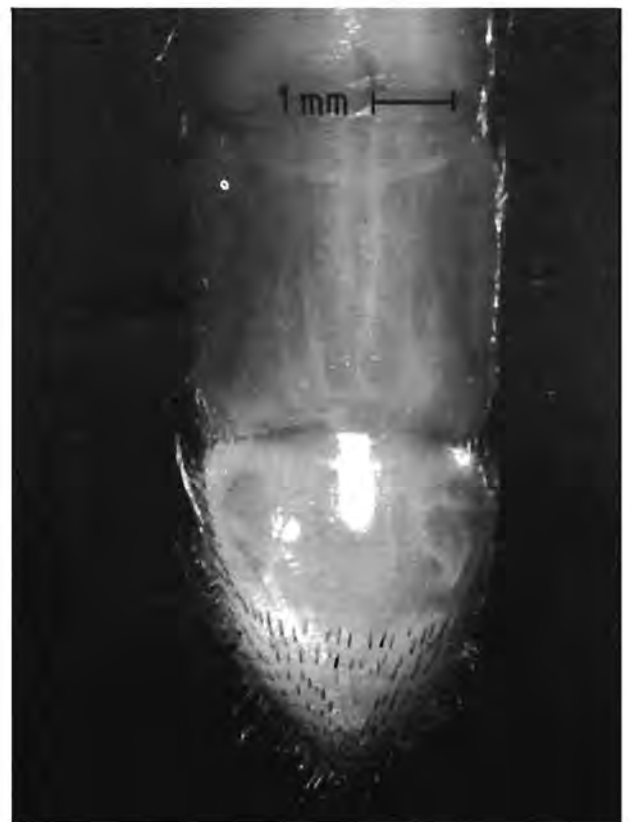


PLATE 2: Ninth abdominal tergites of hybrid and non-hybrid larvae showing intermediate form of tergite of hybrid larva.

a) *Onymacris rugatipennis rugatipennis*, with rounded apex, short setae and anterior bare part three times longer than apical setose part;



c) *Physadesmia globosa*, with pointed apex, long setae and anterior bare part more or less the same length as apical setose part.

b) hybrids from reciprocal crosses between (a) and (c) showing i) intermediately rounded apex, short setae and anterior bare part two times longer than apical setose part and ii) intermediately rounded apex, short setae and anterior bare part three times longer than apical setose part;
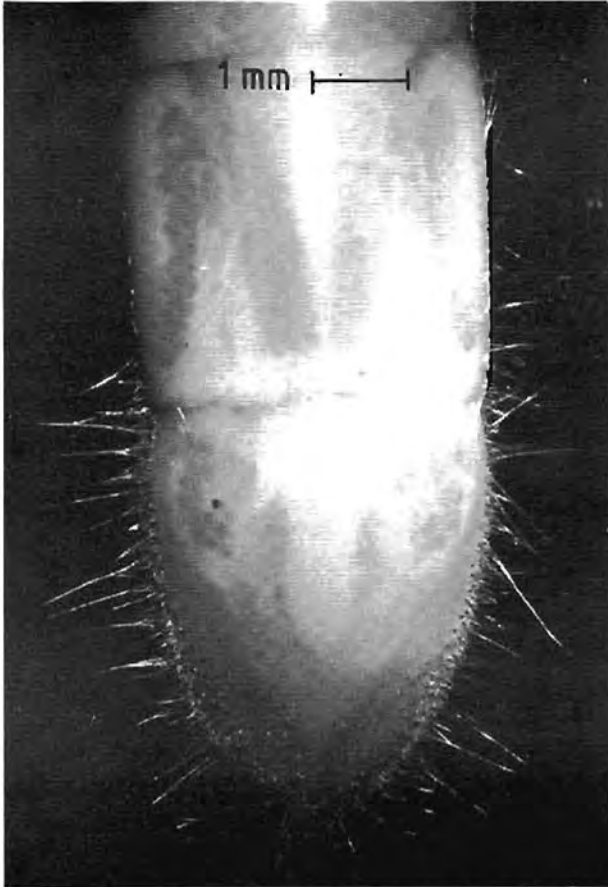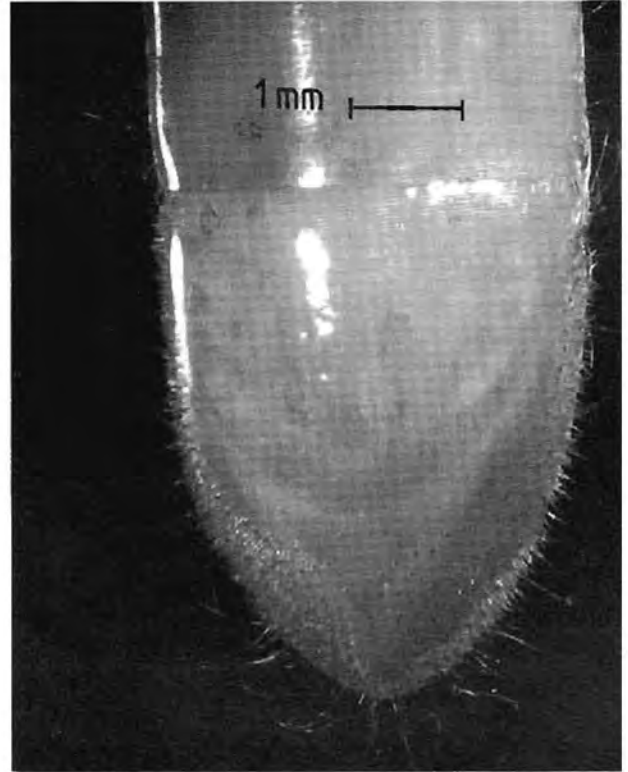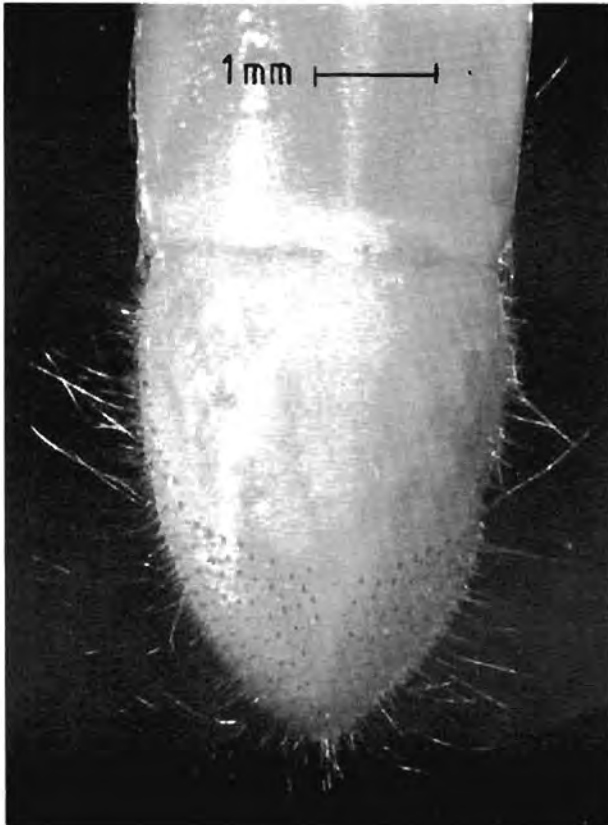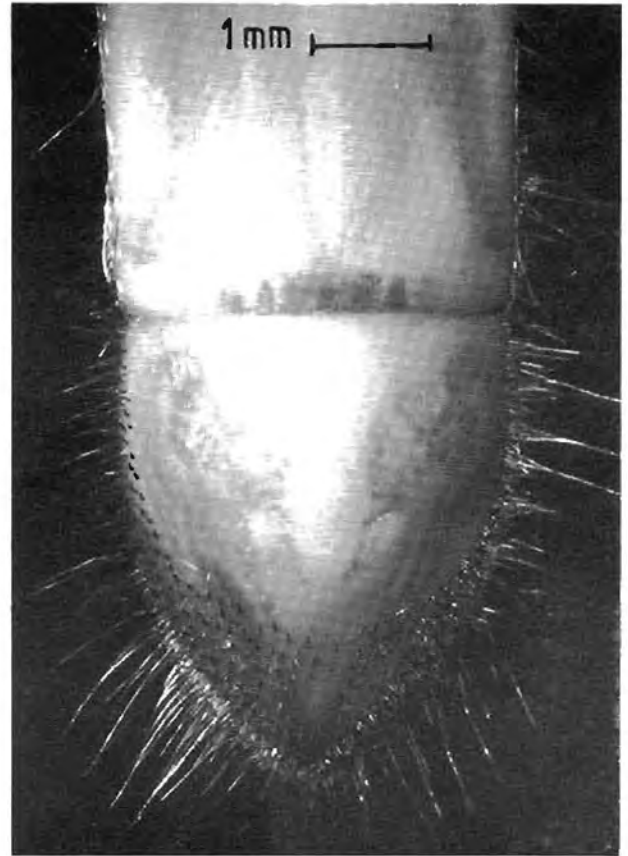
PLATE 3. Ninth abdominal tergites of hybrid and non-hybrid larvae showing intermediate form of tergite of the hybrid larva.

a) *Onymacris rugatipennis rugatipennis*, with rounded apex, short setae and anterior bare part three times longer than apical setose part;





c) *Onymacris rugatipennis albotessellata*, with pointed apex, longer setae and anterior bare part two times longer than apical setose part.

## 3.3 Spermatozoa

Spermatozoa were found to be present in the spermathecal gland of every female individual that was dissected including those that had been isolated from males for a period of ten months. The spermatozoa in the spermathecal glands of recently mated females (i.e. where copulation had occurred the day before the beetle was dissected) as well as in females that had been isolated for ten months, appeared to be completely inactive.

## 4 DISCUSSION

### 4.1 The production of infertile eggs by isolated females

In most insects the spermatozoa received by the female at a single mating remain alive in the spermatheca — perhaps nourished by secretions from associated glands (Davey, 1965) — and fertilize eggs throughout the oviposition period. In a few insects, however, the sperm which are not immediately used for fertilization are digested and absorbed by the female (Wigglesworth, 1972).

In the tenebrionid species used in this study, sperm received at one copulation apparently sufficed for egg fertilization for a maximum of ten weeks, after which time all eggs produced were inviable. It was thought, therefore, that these females may be digesting excess sperm. However, spermatozoa were found in the spermathecal glands of isolated females which had produced only inviable eggs for a period of up to eight months, indicating that not all excess sperm were absorbed.

One possible explanation may be that, among the tenebrionids used in this study, the spermatozoa that were stored in the spermathecae, though nourished and viable, were not used for fertilization of the eggs. De Villiers (1984) has found that in the Namib desert tenebrionid *Onymacris unguicularis* (Haag), the spermatophore is retained in the bursa of the female and most of the spermatozoa probably remain inside it. It is therefore likely that the bursal spermatozoa are responsible for fertilizing the eggs and not the spermathecal gland spermatozoa. When the bursal sperm supply is depleted, absorbed, or no longer viable due to the limited supply of nutrients donated with the spermatophore by the male, then infertile eggs will be produced. This occurs despite the presence of apparently normal spermatozoa in the spermathecal gland which are provided with an indefinite supply of nutrients and are therefore able to remain alive for much longer periods of time.

## 4.2 Breeding experiments

In many insects a second copulation, in some way, prevents further use of the previous spermatozoa. De Villiers (1984) has found that in *O. unguicularis* the new spermatophore obtained at each copulation replaces the old spermatophore, which is then ejected by the female, presumably with its remaining contents of old spermatozoa. Although some of the old spermatozoa may still be present in the spermathecal gland, it is thought that the majority of spermatozoa fertilizing the eggs come from the new spermatophore, and consequently, the spermatozoa from the most recent copulation have the greater opportunity to fertilize the eggs. In the crossing experiments using females that had been isolated for ten weeks, one would therefore expect spermatozoa donated by the most recent copulation (in this case intergenerically and intersubspecifically mated females) to have the advantage in fertilizing the eggs. The period of isolation to which the females were subjected prior to crossing experiments may, therefore, have been an unnecessary precaution.

In most insects mating has the effect of increasing egg production (Hinton, 1981 Wigglesworth, 1972). In addition, in some beetles male pheromones are known to influence the egg maturation of females (Crowson, 1981). In this study, all the females that were isolated from males produced significantly fewer eggs than the control females which were in contact with conspecific males. Egg production, may therefore possibly be under pheromonal control, or may be stimulated by impregnation or some combination of the two.

With the exception of the *O. r. rugatipennis* x *P. cribripes* cross, all females that had previously been isolated, showed an increased egg production following the introduction of a non-conspecific male. It would seem, therefore, that the stimulus for increased egg production is, in these cases, provided even if the male is not conspecific.

The lack of an increased egg production by females used in the *O. r. rugatipennis* x *P. cribripes* cross, in whichever direction it was run, indicated that if impregnation is required as a stimulus, then the infrequent attempts at mounting did not end in successful copulation. If, alternatively, a pheromonal stimulus for increased egg production is required, then the stimulus provided by the non-conspecific male is inadequate. This might be expected, however, due to the species specificity of most pheromones.

Since they appear unable to respond to each others' mating signals, it can therefore be assumed that hybridization between *O. r. rugatipennis* and *P. cribripes* will never occur in nature, even although their ranges overlap in the Kuiseb River bed.

One problem in dealing with non-virgin individuals, lies in the possibility that they will produce eggs fertilized by spermatozoa aquired prior to experimental matings (Parker, 1970). However, in this case this possibility can largely be ruled out.

Field captured and virgin *P. globosa* females mated to *O. r. rugatipennis* males continued producing only in-

viable eggs, in spite of the male presence resulting in an increased egg production. This suggests that old spermatozoa remaining from previous copulations, were incapable of, fertilizing the eggs. If intergeneric mating did occur in this cross (as is suspected from the increased egg production response) then either the foreign spermatozoa were incapable of fertilizing the eggs or, due to genetic incompatibility, the zygote was unable to develop to first instar stage.

All *O. r. rugatipennis* females mated to *P. globosa* males produced viable eggs which hatched into larvae that were intermediate between both parental types. The resulting larvae, therefore, were thought to be hybrids and not offspring from previous copulations. Since the hatching success of hybrid eggs resultant from this intergeneric cross was not significantly different from that of the eggs produced by control females, it can be assumed that there is no genetic incompatibility which would cause a reduction in the number of zygotes capable of developing to first instar stage. Older larvae resultant from this cross were, however, smaller than non-hybrid larvae of the same age and there is a possibility that they are genetically less well equipped for their environment and/or inherently weaker than the non-hybrid larvae.

In view of the fact that courtship between these two sympatric species appears to be ambivalent — males of both species frequently courting females of the other species — and that intergeneric mating between them results in hybrid larvae, it would appear that hybridization between these two taxa should be a common occurrence. Despite extensive pitfall trapping in the area, only two adult hybrid individuals have been found. This suggests that either very few larvae reach adulthood, or alternatively, mating is not as ambivalent as courtship appears to be.

The failure of the hybrid larvae to reach adulthood prevents us from establishing whether the morphologically intermediate individuals found in the wild and hybrids reared in the laboratory were structurally similar. Although other evidence strongly suggests the possibility of natural hybridization between these two groups, breeding experiments will have to be continued before sound empirical evidence can be provided.

If a large majority of hybrid larvae do reach adulthood, then only a rarity of intergeneric mating would account for the rarity of hybrids resultant from this cross. We suggest that the superficially ambivalent courtship exhibited by these two groups may also include the use of an attractant pheromone. This pheromone may be similar enough in both species, to allow some intergeneric attraction, as is frequently observed in the wild. It is expected, however, that intergeneric mating would not occur except in the absence of conspecific mates. Since both species are particularly abundant in the Kuiseb River bed and since they overlap in their use of time, one would expect an abundance of both sexes of both species during the activity

period and hence little intergeneric mating, which possibly accounts for the extreme rarity of these hybrids.

Mating between *O. r. rugatipennis* and *O. r. albotessellata* resulted in larval offspring that were morphologically intermediate between both parental types. The high egg hatching success and readiness of the adults to mate intersubspecifically indicates that these two taxa are fully interfertile in the laboratory, which is to be expected of subspecies. However, by definition, subspecies are described as being fully interfertile but geographically isolated populations of the same species which have developed unique distinguishing characters. While *O. r. rugatipennis* and *O. r. albotessellata*, are fully interfertile under laboratory conditions, the populations are not geographically isolated. Their ranges are contiguous on the south bank of the Kuiseb River, where dune and river bed habitats overlap. Although *O. r. albotessellata* is rare in the river bed, it still occurs there in sufficient numbers to allow introgression.

Beetles were not reared to adulthood in the crossing experiments carried out in the laboratory, thus it is not known what characters the offspring of these crosses would exhibit in the adult phase. However, whether the characters distinguishing *O. r. albotessellata* from *O. r. rugatipennis* are multigenic, or under the influence of a major gene, introgression would lead to the production of at least two adult forms in the following generations of both populations *viz* the *O. r. rugatipennis* form and the *O. r. albotessellata* form or a range of intermediate forms.

Since both populations are highly mobile within their preferred ranges — one would expect introgression at the interface of their two ranges to result in the appearance of both adult forms, or a range of intermediate forms, away from as well as within the zone of overlap i.e. in the river bed and in the dunes. This however, is not the case. While both adult forms are found in the eastern reaches of the river, neither the *O. r. rugatipennis* form, nor any intermediate forms, have, to our knowledge, been found far into the dunes. Since there is no obvious variation in the morphology of *O. r. albotessellata* in its own habitat it is suggested that interbreeding does not in fact occur in the zone of overlap. The fact that they are fully interfertile in the laboratory by no means implies that interbreeding occurs in the field. A closer investigation into their courtship and breeding activities, in the field of positive assortative mating, in the natural habitats of each of the two forms may lead to their reclassification as species.

## 5 SUMMARY

Mating was found to occur in all crosses, except between *P. cribripes* and *O. r. rugatipennis* and was coupled with an increased egg production which may have been the result of impregnation or simply of a male pheromonal stimulus. Intergeneric hybridization be-

tween *O. r. rugatipennis* females and *P. globosa* males was shown to be genetically possible. None of the hybrid larvae were reared to adulthood however, and it is not known whether laboratory reared adult hybrids would have resembled the morphologically intermediate individuals found in the wild, thus providing evidence for their assumed hybrid origin. Crosses carried out in the reverse direction were unsuccessful presumably due to the failure of the foreign male gamete to fertilize the egg or failure of the zygote to develop to first instar.

The rarity of intergenetic hybridization in the wild in spite of apparantly ambivalent courtship and the genetic possibility of hybridization, could be due to very few hybrid larvae reaching adulthood or because mating between these two species is induced by species specific pheromonal attraction. Although, intergeneric attraction was often observed in the field, intergeneric matings were not expected to occur in the wild except in the absence of conspecific mates. This is unlikely to occur due to the high degree of overlap in the use of both time and space by both species.

It is thought that *O. r. rugatipennis* and *O. r. albotessellata* should be separated at more than the subspecific level since, although fully interfertile in the laboratory, there is no evidence of introgression in the field even though their ranges overlap.

# 6 ACKNOWEDGEMENTS

# 7 REFERENCES

CROWSON, R.A.
    1981: *The Biology of the Coleoptera.* Academic press, New York.

DAVEY, K.G.
    1965: *Reproduction in the Insects.* Oliver and Boyd, Edinburgh.

DE VILLIERS, P.
    1984: Reproductive strategy of the Namib tenebrionid, *Onymacris unguicularis* (Haag). Unpublished Ph.D. thesis. University of the Witwatersrand, Johannesburg.

GRAY, A.P.
    1972: *Mammalian hybrids.* 2nd ed. Commonwealth Agriculture bureaux.

HAMILTON, W.J. III., BUSKIRK, R.E. and BUSKIRK, W.H.
    1976: Social organization of the Namib desert tenebrionid beetle, *Onymacris rugatipennis. Can. Ent.* **108**: 305—316.

HAMILTON, W.J. III. and PENRITH, M-L.
    1977: Description of an individual possible hybrid tenebrionid beetle and the habitat preference of the parental species. *Can. Ent.* **109**: 701—710.

HINTON, H.E.
    1981: *Biology of Insect Eggs.* Vol. 1. Oxford, Pergamon press.

PARKER, G.A.
    1970: Sperm competition and its evolutionary consequences in the insects. *Biol. rev.* **45**: 525—567.

PENRITH, M-L.
    1975: The species of *Onymacris* Allard (Coleoptera: Tenebrionidae). *Cimbebasia* **4**: 47—97.

PENRITH, M-L.
    1979: Revision of the Western Southern African Adesmiini (Coleoptera: Tenebrionidae). *Cimbebasia* **5**: 1—94.

SCHULZE, L.
    1962: The Tenebrionidae of Southern Africa XXXIII Descriptive notes on the early stages of *Onymacris rugatipennis* (Haag) and *Lepidochora discoidalis* (Gebien) and keys to genera and species of Adesmiini and Eurychoriini. *Sci. pap. of the Namib Desert Research Station.* **7** (x).

SCHULZE, L.
    1964: The Tenebrionidae of Southern Africa XXXIX — a revised key to the larvae of *Onymacris* Allard (Coleoptera: Adesmiini). *Sci. pap. of the Namib Desert Research Station.* **23** (vi).

WIGGLESWORTH, V.B.
    1972: *The Principles of Insect Physiology* 7th ed. Cambridge University press.