



Species Isolation, Genital Mechanics, and the Evolution of Species-Specific Genitalia in Three Species of Macroductylus Beetles (Coleoptera, Scarabeidae, Melolonthinae)

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SPECIES ISOLATION, GENITAL MECHANICS, AND THE
EVOLUTION OF SPECIES-SPECIFIC GENITALIA IN
THREE SPECIES OF *MACRODACTYLUS* BEETLES
(COLEOPTERA, SCARABEIDAE, MELOLONTHINAE)

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Abstract.—The question asked was why male genitalic structures have diverged in three syntopic species of *Macrodactylus* beetles. Four hypotheses were evaluated: 1. The ways in which male genitalia mesh with internal female structures indicate that selection for species isolation via mechanical exclusion (“lock and key”) is unlikely to explain the genitalic differences. 2. The specific mate recognition hypothesis also clearly fails to explain genitalic differences due to the implausibility of postulated environmental effects on genitalia, and lack of postulated coevolution of male and female morphologies. 3. Selection for species isolation via differences in genitalic stimulation (sensory lock and key) is unlikely due to relatively infrequent cross-specific pair formation and intromission in the field, and “excessive” numbers of species-specific genitalic structures and male courtship behavior patterns which nevertheless occasionally fail. It also fails to explain the frequent failure of *intraspecific* copulations to result in sperm transfer. This hypothesis cannot, however, be rejected as confidently as the previous hypotheses. 4. Conditions under which sexual selection by cryptic female choice could take place are common. Females frequently exercise their ability to prevent sperm transfer by conspecific males even after intromission has occurred, and females generally mate repeatedly, probably with different males. Males behave as if cryptic female choice is occurring, courting assiduously while their genitalia are within the female. Sexual selection by female choice could thus contribute to the divergence in genitalic structures.

Key words.—Beetles, cryptic female choice, genitalic evolution, lock and key, species isolation, specific mate recognition.

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Species-specificity in reproductive structures and behavior has often been thought to have evolved under selection to avoid cross-specific pairings (e.g., Mayr, 1963). Alternative explanations for species-specificity have also been proposed, however, including specific mate recognition (Paterson, 1985), and sexual selection (West-Eberhard, 1983; Eberhard, 1985).

The genitalia of animals with internal fertilization are often species-specific in form (Eberhard, 1985). Possible reasons for differentiation in genitalic morphology include two forms of the species isolation hypothesis: selection to prevent mechanical mesh between species (“lock and key”) (e.g., Shapiro and Porter, 1989); and selection to provide the female with species-specific stimulation (sensory lock and key) that allows her to recognize and avoid cross-specific males (DeWilde, 1964). The specific mate recognition and sexual selection hypotheses represent further alternative explanations of genitalic evolution.

One of the problems in comparing the species isolation hypotheses with others is that most quantitative studies of pairing preferences have been done in captivity (e.g., Spieth and Ringo, 1983; Verrell and Arnold, 1989; Krebs and Markow, 1989), since it is usually difficult to quantify the relative frequency of cross-specific pairings in nature (for an exception, see Newberry and Brothers, 1990). The animals of this study, three species of scarab beetles in the rosechafer genus *Macrodactylus*, offer an opportunity to at least partially overcome this problem. They occur in substantial numbers, often in mixed aggregations of two or three species. Pairs are common, and often remain together for many hours. Females emerge from underground with the start of the rainy season, feed until their eggs are mature, then oviposit underground. Some females appear to then emerge again above ground to undergo a second gonotrophic cycle.

The male genitalia of *Macrodactylus* bee-

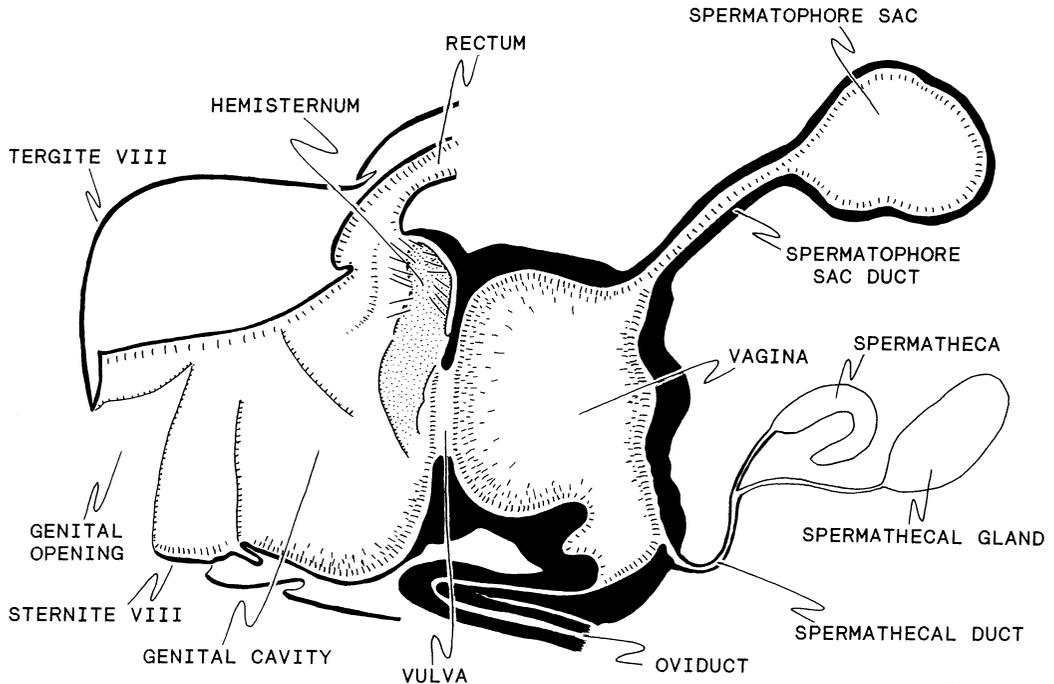


FIG. 1. Schematic cutaway view of the lower reproductive tract of a female *M. sericinus* with genital aperture open. The only rigid structures in the tract are the hemisternum and (to a lesser extent) the spermatheca.

cles are species-specific in form (Carrillo and Gibson, 1960), and may be the most versatile ever studied. They take on at least four different configurations within the female, and appear to perform at least five and perhaps up to eight different functions during courtship and copulation: some structures appear to provide courtship stimuli; some seem designed to help the male force his genitalia deeper into the female; and some participate directly in sperm transfer (Eberhard, 1992). The question posed here is what conditions are likely to have been responsible for the divergence in the genitalic morphologies of these species.

Basic Genitalic Morphology

The following is summarized from a detailed account of the genitalic morphology of these species of *Macroductylus* (Eberhard, 1992). The external female genitalic opening is formed by the sternite and tergite of segment VIII, and leads to a membrane-lined genital chamber (Fig. 1). The interior wall of this chamber bears a pair of sclerites, the hemisternites, whose dorsal portions are free and flap-like. Between and below the

hemisternites is the vulva, an opening leading inward into the vagina. The vulva and the walls of the vagina are heavily muscled, while the hemisternites are attached only in their basal half and are not associated with major muscles. The duct to the spermatophore sac, where the female stores and degrades spent spermatophores, opens on the dorsal wall of the vagina. Spermatophores are relatively small, on the order of 0.1–1% of the male's live weight.

Copulation involves two stages of intromission: entrance of the male's parameres and the distal portion of his phallobase into the female's genital chamber; and then eversion of his internal sac through the vulva and into the vagina, where a spermatophore is formed and then inserted and held in the entrance of the spermathecal duct. In some specimens frozen in copula, the male genitalia had penetrated only as far as the genital chamber, and the vulva was closed and the vagina walls squeezed together, probably making intromission through the vulva into the vagina difficult or impossible for the male.

The male genitalia of the three species

differ in several respects. The highly sclerotized parameres have different sizes and forms. During copulation the distal portions of the parameres rest against the female's hemisternites in her genital cavity. The form of the hemisternites also differs between the three species, and the differences are mechanically appropriate to mesh with the parameres of the male (e.g., in *M. sericinus* a rounded prominence on the distal portion of the male's paramere fits into an indentation in the female's hemisternite).

Copulation often lasts for 30 min or more in *Macrodactylus*. Even after >30 min of intromission in captivity, males usually had not transferred a spermatophore: spermatophores had not yet been made in 9 of 10 *M. costulatus* pairs and 10 of 11 *M. sericinus* pairs when they were sacrificed >30 min after copulation began.

There are species differences in the "spermatophore insertion" and "fingers" configurations of the male's membranous internal sac when it is everted in the female's vagina. In none of the species is there any sign in the soft and flexible vagina wall of a structure which would correspond to species differences in the males, nor of structures which could mechanically block male structures of another species. Thus differences in male internal sac characters will not mechanically preclude cross-specific entry of male genitalia into the vagina.

Still another male structure whose morphology differs in the three species is the spermatophore. The area of the female which the spermatophore contacts is soft and featureless. Once again there are no differences in female morphology corresponding to differences in spermatophores, much less differences which could mechanically block insertion of a cross-specific spermatophore.

MATERIALS AND METHODS

All field surveys of mated pairs and observations of behavior were made near San Antonio de Escazu, San José Province, Costa Rica, in May and June of 1988 to 1990 at the start of the rainy season when adults of all three species are active.

Field observations of solitary males encountering other beetles were made at aggregations of beetles on *Citrus* sp., *Croton*

sp., *Phenax* sp., and *Vernonia* sp. plants, either by following free-ranging males, or by placing small broken branches in the paths of such males, allowing them to climb onto them, and then moving the branches and allowing the males to climb off onto another branch where other beetles were present. The frequencies of encounters with different types of beetles reported in the results section thus do not necessarily reflect relative frequencies of such encounters in nature.

Observations of pairing behavior in captivity were made during the afternoon using beetles which had been kept captive at least 24 hours in unisexual groups on cuttings of the foodplant on which they were originally collected. A female of each of two species and then a male of each of these species were introduced into a small jar (125 cc) which contained a hardware cloth support on which the beetles could climb. About 20 jars of beetles were set up on a given afternoon, and I checked each jar every four to seven min for one hour to determine whether either male had mounted a female (with his front legs clasping her thorax), and whether any pairs were copulating.

RESULTS

Occurrence on Different Plant Species

The beetle species differed with respect to the spectrum of plant species on which they occurred. Of the six plant species most thoroughly sampled (with largest accessible populations of beetles), *Citrus* spp. (Rutaceae), *Vernonia* sp. (Compositae), and *Acnistus arborescens* (Solanaceae) had only *M. costulatus* and *M. sylphis*. In contrast, only *M. costulatus* and *M. sericinus* were found on *Phenax* sp. (Urticaceae) and *Croton* sp. (Euphorbiaceae) (Table 1). Only on *Eriobotrya japonica* (Rosaceae) were all three species found together on the same plants.

Relative numbers of beetles varied on different plant species that harbored the same pair of beetle species. For instance, relations between numbers of *M. costulatus* and *M. sylphis* were about 1:5 on *Citrus* ($N = 839$), but about 5:1 on *Vernonia* ($N = 435$). Relations between *M. costulatus* and *M. sericinus* were about 1:4 on *Phenax* ($N = 1618$), but about 30:1 on *Croton* ($N = 250$) (beetle species differ in distribution among

TABLE 1. Numbers of paired and solitary individuals in samples from different species of plants (N = number of plants sampled; only on *Vernonia* was it possible to count all beetles on a given plant).

Beetles	Plants					
	<i>Citrus</i>	<i>Phenax</i>	<i>Acnistus</i>	<i>Vernonia</i>	<i>Croton</i>	<i>Eriobotrya</i>
<i>M. costulatus</i>						
Pairs	50	125	69	190	99	9
Sol. males	18	64	34	31	38	6
Sol. females	18	14	25	36	7	3
<i>M. sylphis</i>						
Pairs	184	0	21	28	0	40
Sol. males	184	0	42	13	0	38
Sol. females	151	0	21	19	0	8
<i>M. sericinus</i>						
Pairs	0	471	0	0	1	3
Sol. males	0	244	0	0	2	2
Sol. females	0	104	0	0	3	1
Cross-specific pairs						
<i>M.c.</i> × <i>M. ser.</i>	0	10	0	0	1	0
<i>M.c.</i> × <i>M. syl.</i>	0	0	0	1	0	0
<i>M. ser.</i> × <i>M. syl.</i>	0	0	0	0	0	0
N	12	18	12	25	3 ¹	9

¹ One "individual" tree was a series of five tightly interdigitating trees.

plant species, $P \ll 0.001$; Chi squared $> 3,000$, $df = 12$).

Behavior in the Field

Males walking on vegetation usually responded to conspecific females they encountered by attempting to mount them, but attempted to mount cross-specific females much less often (Table 2). Attempts to mount conspecific females frequently led to the male clasping the female with his front legs, and in 29.3% of 123 cases to intromission within five min. In contrast, all of the 37 cross-specific attempts to mount were relatively brief, and none resulted in intromission or even in the male clasping the female with his front legs for more than five sec ($P < 0.001$; Chi squared = 13.9, $df = 1$). In most cases the male desisted spontaneously and walked away.

TABLE 2. Frequencies of attempted mounting by males when they encountered conspecific and cross-specific females in the field (sample sizes in parentheses).

Males	Females		
	<i>M. costulatus</i>	<i>M. sylphis</i>	<i>M. sericinus</i>
<i>M. costulatus</i>	71% (21)	15% (13)	25% (4)
<i>M. sylphis</i>	0% (13)	76% (70)	—
<i>M. sericinus</i>	14% (7)	—	63% (32)

Since mounted males often rode females for extended periods (up to several days in the field), it was possible to obtain a second, larger sample of data on the frequencies of conspecific and cross-specific pairings in the field by counting the numbers of pairs in which males rode on the female's dorsum, clasping her thorax (Table 3). In all three species cross-specific pairings represented less than 2% of the pairings in which a female of that species participated.

A final criterion for the relative frequency

TABLE 3. Numbers of conspecific and cross-specific pairs seen in nature. Only observations from plants ($N = 65$) where at least two species of beetle were present are included, so these may be overestimates of the frequency of cross-specific pairing.

Males	Females		
	<i>M. costulatus</i>	<i>M. sylphis</i>	<i>M. sericinus</i>
<i>M. costulatus</i>	544	2	8
<i>M. sylphis</i>	0	324	0
<i>M. sericinus</i>	1	1	507
Percent of paired females which were paired with male of another species			
	0.18%	0.90%	1.60%
Percent of all females (solitary and paired) paired with a male of another species			
	0.12%	0.54%	1.46%

TABLE 4. Characteristics of samples of females of *M. costulatus*, *M. sericinus*, and of a probable hybrid. The probable hybrid resembles *M. sericinus* in two characters, and is intermediate in two others (see also Fig. 2).

	Tergite VIII			Tergite VIII		Bristles of tergite VIII limited to distal third?		Elytra setose?	
	Black	Brown	Orange	Flat	Domed	Yes	No	Yes	No
<i>M. costulatus</i>	25	0	0	25	0	25	0	25	0
<i>M. sericinus</i>	0	0	25	0	25	0	25	0	25
Hybrid		1		intermed.			1		1

of cross-specific matings in the field is the frequency of hybrid individuals. One female which was intermediate in several respects between *M. costulatus* and *M. sericinus* (Table 4, Fig. 2) was collected in a mixed aggregation of these two species. Several hundred females of both *M. costulatus* and *M. sericinus* were collected in total, giving a hybridization rate of <1%. It is, however, difficult to evaluate the precision of this estimate. It may underestimate hybridization frequencies because of lower survival of hybrids; or it may overestimate them, since many other females of both *M. costulatus* and *M. sericinus* were seen in the field but not counted, and little attention was paid to those plants on which only one species was present. No apparent hybrids were found between *M. costulatus* and *M. sylphis*, or between *M. sylphis* and *M. sericinus*.

Some conspecific pairings in the field that resulted in intromission almost certainly did not result in sperm transfer, as they were relatively brief. At least 75% (data were incomplete in two cases) of 16 intromissions in conspecific *M. sericinus* pairs were short (<two min—some were less than five sec); the corresponding frequencies of short copulations were at least 18% for 11 *M. sylphis* (data were incomplete in four cases), and 0% for 6 *M. costulatus* (data were incomplete for two cases).

Terminations of brief copulations were initiated by the male, without overt signs of resistance from the female. The male withdrew his genitalia, often turned 180° on the female's dorsum, sometimes turned another 180° and made another brief intromission, and then climbed off and walked away. When five female *M. sericinus* were frozen within five sec after the male withdrew his genitalia, it was found that in all cases the female's vulva and vaginal walls were strongly contracted, thus probably prevent-

ing the male's genitalia from penetrating past her genital chamber and entering her vagina where sperm transfer takes place (Eberhard, 1992). Such contraction was never seen in 21 other females frozen when not copulating.

Behavior in Captivity

Beetles kept in jars with individuals of other species showed a strong tendency to form conspecific pairs (Table 5). Intromission may have also been more common in conspecific (total of 52% of 233 pairs) than cross-specific pairs (total of 0% of 7 pairs) (Table 5), although numbers of cross-specific pairs were too small to permit statistical tests by species.

Short copulations (<two min) by *M. sericinus* were less common in captivity than in nature. The male withdrew his genitalia within the first 15 min in 27% of 15 copulations in captivity (75% withdrew within the shorter time period of two min in the field— $P < 0.001$; Chi squared = 7.24, $df = 1$). Frequencies for *M. sylphis* showed a similar, though statistically insignificant trend (3% of 31 within 15 min in captivity; 18% within two min in the field).

Duration of courtship prior to intromission was also longer in captive pairs. The male had failed to achieve intromission three min after the pair formed in 53% of 109 conspecific captive pairs of *M. costulatus*; this contrasts with 12% of 8 pairs in the field ($P = 0.05$; $G = 3.8$, $df = 1$ with G Test using Yates' correction for continuity).

DISCUSSION

Species Isolation Hypotheses

Since cross-specific pairings occur in nature, and there is even occasional hybridization between at least one pair of species, it might seem that differences in sexual behavior and morphology evolved as species

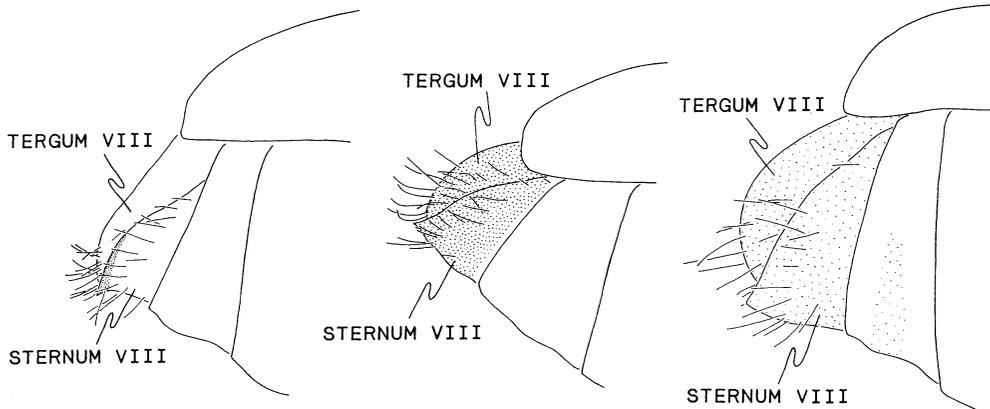


FIG. 2. Lateral view of posterior portion of abdomen of *M. costulatus* female (left), *M. sericinus* female (right), and a probable hybrid female (center). The colors of the stippled portions of tergite and sternite VIII range from dark brown (darkest stippling) to tan (lightest stippling). Nonstippled portions of tergites and sternites VIII are black.

isolation mechanisms to minimize such pairings. The data from this study indicate, however, that this function is unlikely, at least for the genitalic characters.

In the first place, behavioral observations indicated that avoidance of cross-specific pairings occurred in the majority of cases before genital contact occurred. The frequency with which a female was paired in the field with a cross-specific rather than a conspecific male ranged between 0.2 and 1.6% (Table 3), and these numbers may be overestimates since they do not include bee-

bles found on plants where only a single species was present. For instance, large numbers of *M. costulatus* occur in monospecific aggregations on *Prunus persica* at higher elevations in Costa Rica (A. Solis, pers. comm.).

Laboratory observations (Table 5) suggested additionally that in only a small fraction of cross-specific pairs does the male introduce his genitalia into the female. Thus the context in which species identification via genitalic stimuli could occur is rare, probably in substantially less than 1% of all copulations in all three species.

The relative lack of opportunity in nature for selection favoring species isolation to act on genitalia constitutes an important test of the species isolation hypotheses, but does not justify their outright rejection for reasons that will be discussed separately for each of the two forms of the hypothesis.

I. Mechanical Lock and Key

The lock and key hypothesis would hold that genitalic differences between these species evolved to mechanically prevent cross-specific matings, as may occur in some species pairs or their hybrid offspring (e.g., Charlton and Cardé, 1990; Kubota, 1988; see also Krebs and Markow, 1989). Shapiro and Porter (1989) argue that it is difficult to disprove the lock and key hypothesis using data on present day functions because the species-isolation function is likely to be evo-

TABLE 5. Pair formation (male mounts female and clasps her with his front legs) and copulation in captivity. Pair formation was registered only if all four beetles in the container were unpaired during the preceding observation period. Since pairs sometimes separated, the number of pair formations is greater than the number of replicate containers.

	Experiment		
	<i>M. costulatus</i> + <i>M. sylphis</i>	<i>M. costulatus</i> + <i>M. sericinus</i>	<i>M. sylphis</i> + <i>M. sericinus</i>
Mixed pairs/con-specific pairs	0/125	6/93 ¹	1/15 ²
Rate of copulation/pair			
Conspecific	71.2%	23.7%	46.7%
Mixed	—	0%	0%
Number of replicate containers	104	58	11

¹ Cross-specific pairs involved five *M. costulatus* males, one *M. sericinus* male.

² Cross-specific pair involved a *M. sylphis* male.

lutionarily transient. Species-specific genitalic differences may not function to isolate species in many contemporary populations, even though they did so in the past. Genitalic isolating mechanisms are expected to be superseded in evolution by other, more advantageous mechanisms (such as courtship behavior) which come into play before genitalic coupling occurs and result in less wasted time and effort.

The lock and key argument can be rejected for the beetles of this study, however, because there is simply no female "lock," vestigial or otherwise, that could mechanically exclude male genitalia on the basis of their species-specific morphology (Eberhard, 1992). The male's internal sac, which shows a variety of differences between species, is everted inside the female vagina, the walls of which are soft, extensible, and nearly featureless in all three species. A soft, pliable "key" is inflated inside a soft, pliable, and relatively featureless "lock." It is true that the vagina can contract as a unit, and can thus probably prevent the male from everting his internal sac through the vulva. But it is mechanically incapable of excluding one form of internal sac but admitting another simply on the basis of mechanical fit. Females also lack structures capable of excluding cross-specific spermatophores on the basis of mechanical fit.

There is one female genitalic structure (the hemisternite) which is relatively rigid and which shows species differences that are correlated with differences between male genitalic structures (the parameres). But the hemisternites are not placed in such a way that they can constitute physical barriers to deep intromission. The hemisternites are connected to flexible membranes, and their dorsal portions (which contact the species-specific distal portions of the male parameres) are flap-like, lying free in the genital chamber (Fig. 1). They are located above and to the side of the vulva rather than over it (Fig. 1), and are displaced dorsally and anteriorly within the female's body during copulation (Eberhard, 1992). The male's parameres will lie less flush against the dorsal portions of the female's hemisternites in cross-specific pairings, but this in and of itself is neither a physical barrier to deeper intromission, nor an impediment to the

male's maintaining his genitalia securely in place in the female's genital chamber.

II. Sensory Lock and Key

Females might recognize males as conspecifics by the stimuli produced by species-specific male genitalic structures (DeWilde, 1964). The complementary fit between male parameres and female hemisternites (which bear numerous setae that could act as sense organs), the presence of fringing bristles on the parameres of some *Macrodactylus* species (Carrillo and Gibson, 1960), and the apparent ability of females to exclude males from the deeper penetration necessary for sperm transfer are all in agreement with this idea. The "ghosts of species isolation past" argument of Shapiro and Porter (1989) could apply here. Perhaps, even though selection for species isolation is not responsible for maintenance of species-specific genitalic traits in present day populations, the species isolation context was common enough in the past to result in selection for species specificity in male genitalia. This possibility cannot be discarded with complete confidence, but there is one weak and one relatively strong reason to doubt it: 1) Selection for species isolation should be stronger in pairs of species which more often form cross-specific pairs. At present the most common cross-specific pairing is *M. costulatus* × *M. sericinus*. Assuming that this was also true in the past, the species isolation hypothesis would predict greater genitalic differences between these two species. This is not borne out by the data. The parameres of *M. sericinus* are especially different from those of *M. costulatus* and *M. sylphis*, but the "spermatophore insertion" and "fingers" configurations of the internal sac and the spermatophores of *M. sylphis* are especially distinctive compared with those of the other two species. These results do not represent strong evidence against the species isolation hypothesis, however, since the derived and ancestral states are not known for any of these characters, the assumption of past frequencies of cross-breeding is weak, and nothing is known of rates of cross-breeding in other parts of the species' ranges. 2) Stronger evidence against the sensory lock and key hypothesis comes from the veritable deluge of species-specific stimuli a

female *Macroductylus* receives during copulation. Working backward in the insemination sequence and from inside the female's body to her external surface, she may be stimulated during courtship and copulation by at least eight different sets of species-specific stimuli: the spermatophore in her vagina; the spermatophore insertion configuration of the male's internal sac in her vagina; the fingers configuration of his internal sac in her vagina; his parameres on her hemisternites; his phallobase and parameres rubbing and tapping on the outer surface of her eighth sternite and tergite; his ventral bristles rubbing on her elytra and tergite VIII; the ventral surfaces of his front legs clasp her thorax; and the rubbing and vibrating movements of his middle legs on her legs and body (Eberhard, 1992). This list may even be an underestimate, since patterns of movements of male structures inside the female are not documented, though they are known to occur (Eberhard, 1992).

On one hand, this seems like an overly long list of "ghosts" of previously functional isolating mechanisms to remain hanging on after males have evolved the ability to reject most nonconspecific females before even mounting them. In addition, these characters would have had to have evolved in more or less this particular order, since signals occurring earlier in courtship and copulation would be supposed by the "ghosts" hypothesis to have evolved to replace ones occurring later in the sequence. Given the likely advantage to females of earlier discrimination (Alexander, 1964; Eberhard, 1985; Shapiro and Porter, 1989) and the evidence given here for relatively simple early recognition cues, it strains one's credulity to accept such an orderly accretion of so many signals. Especially improbable is the relatively large number of different internal signals (four) that would be supposed to have accumulated before the origin of any external signals.

The sensory lock and key hypothesis can still be salvaged by claiming that the multiple male signals are redundant, and represent fail-safe devices. Given the apparent simplicity of early recognition cues actually in use (presumably surface chemicals), the apparently "excessively" large number of

presumed sensory keys in these beetles, and the fact that nevertheless they at least occasionally fail to preclude hybridization, this argument also seems improbable. Also unexplained is the frequent failure of *intra*-specific copulations to result in spermatophore transfer. In sum, these considerations suggest that the sensory lock and key hypothesis does not easily fit the observations, but that it cannot be rejected with complete confidence.

Specific Mate Recognition System Hypothesis (SMRS)

This hypothesis proposes that species-specific characters diverge due to differences in habitat: "... the characters of the fertilization system are adapted to the circumstances impinging on the organism in its natural habitat"; "... a new constellation of adaptive characters can evolve ... [when a population] becomes displaced into, and restricted to a new habitat" (pp. 25–26 of Paterson, 1985; see also Masters et al., 1987). It is difficult, to say the least, to see what habitat differences could possibly have selected for divergence in the form of the male parameres, the fingers and spermatophore insertion configurations of the male internal sac, and the spermatophores of *Macroductylus*, none of which ever emerge from the male's body except while inside the female.

In addition, the SMRS hypothesis would seem to require that species-specific male structures coevolve with changes in female structures (for concrete examples where this has been argued, see Robertson and Paterson, 1982; Zunino, 1987). In the beetles of this study, differences in male paramere form are associated, as predicted, with differences in female hemisternites. However, the differences in two configurations of the internal sac and in the spermatophores are not matched by the predicted corresponding differences in female morphology.

Sexual Selection Hypothesis

The observations reported here were not designed to test the possibility of sexual selection by female choice based on male genitalic characters. They do show, however, that conditions necessary for such selection to occur are common. Females in nature mate repeatedly. Many intromissions, in at

least two of the three species, are aborted by males in nature (perhaps due to the female's failure to open her vulva) before a spermatophore is transferred.

In addition, it is clear that the majority of the sperm transferred when a male successfully passes a spermatophore to a female nevertheless fail to be stored in the female's spermatheca. Females in the field with mature eggs typically contain the vestiges of at least 5 to 10 (and probably more) spermatophores, while the volume of a female's spermatheca is only large enough to hold the sperm contained in 2 to 3 spermatophores (Eberhard, 1992). Some sperm may be digested along with the spermatophores inside the female, since some spermatophores in the sac where they are degraded still contain living sperm (Eberhard, 1992).

Situations in which sexual selection on a male's ability to induce a female to permit deeper intromission may occur in nature in two species of *Macroductylus* at least 10 times more frequently than those in which species recognition via genitalic cues could be advantageous: at least 75% rapid abortion of copulation (<two min) versus 1.6% cross-specific pairing in *M. sericinus*; at least 18% rapid abortions versus 0.9% cross-specific pairing in *M. sylphis*. In addition, dissections of mating pairs of the third species, *M. costulatus*, suggest that the large majority (on the order of 90%) of longer intromissions in captivity also fail to result in spermatophore transfer as also occurs in *M. sericinus* in captivity (Eberhard, 1992).

Thus females are in position to exercise "cryptic female choice" (Thornhill, 1983) at two and perhaps more stages of copulation. The frequencies of these selective contexts are high, and they probably reoccur inexorably generation after generation under a wide variety of environmental conditions (West-Eberhard, 1983). The fact that male *Macroductylus* court assiduously for many minutes at a time while their genitalia are within the female (often producing up to three and four different types of stimuli simultaneously, including head vibrations, leg vibrations, leg rubbing, body vibrations, scraping on the female's dorsum, and genitalic thrusting—Eberhard, 1992) is an additional indication that cryptic female choice occurs in these beetles (Eberhard, 1991).

Field versus Captivity

Many important studies of the possible causes of divergence in courtship behavior and morphology have been performed in captivity (e.g., review by Spieth and Ringo, 1983; Verrell and Arnold, 1989). While differences in the species used in different studies must obviously be taken into account, the data from *Macroductylus* sound a note of caution regarding extrapolation of results from captivity to the field. Both the duration of pre-intromission courtship and the frequency of aborted intromissions differed between field and captivity in at least one species. At least some key aspects of courtship and copulation thus change in captivity. The temptation to make a typological characterization of the courtship and the copulation behavior of a species observed in a single restricted setting should be avoided.

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