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The Lock-and-Key Hypothesis of Mechanical Reproductive Isolation: Variation in Genitalic Fit and its Influence on Spermatophore Transfer in the Grasshopper Barytettix humphreysii

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THE LOCK-AND-KEY HYPOTHESIS OF MECHANICAL REPRODUCTIVE ISOLATION: VARIATION IN GENITALIC FIT AND ITS INFLUENCE ON SPERMATOPHORE TRANSFER IN THE GRASSHOPPER BARYTETTIX HUMPHREYSII

By

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ABSTRACT

THE LOCK-AND-KEY HYPOTHESIS OF MECHANICAL REPRODUCTIVE ISOLATION: VARIATION IN GENITALIC FIT AND ITS INFLUENCE ON SPERMATOPHORE TRANSFER IN THE GRASSHOPPER BARYTETTIX HUMPHREYSII

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The lock-and-key hypothesis predicts that genitalic incompatibility can lead to mechanical reproductive isolation between closely related taxa. Inter-taxon insemination should be impossible when male and female genital morphologies are completely mismatched. But when taxa are incompletely mechanically isolated (as may be characteristic of Barytettix humphreysii races; Cohn and Cantrall, 1974), genitalic incompatiblity should reduce but not entirely eliminate inter-taxon insemination. Insemination success should correspond to the degree of compatiblity between male and female genitalia, regardless of the taxonomic identity of the mated pair.

This study showed that the intra-racial crosses of B. h. humphreysii and B. h. cochisei were characterized by high levels of spermatophore transfer (a measure of insemination success; 84% in both cases). In addition, the genitalia of these crosses showed no major signs of incompatibility in histological serial sections. The curvature of the aedeagus corresponded well with the curvature of the bursal wall, short distances were observed between the apex of the aedeagus and the spermathecal tube opening (i.e. penetration of the dorsal and ventral valves was deep), and there was no unusual rotational instability of the valves. Male and female size differences accounted for the effects of all other genitalic variables on spermatophore transfer, probably as a consequence of the closeness of the genital openings. In addition,

males and females of more similar size were not as successful as males mated to relatively larger females. This data suggested that females may choose among copulating males based on body size differences and the fit of the genitalia (Eberhard, 1985).

In the inter-racial cross, B. h. humphreysii male X B. h. cochisei female, the genitalia appeared to fit together well and spermatophore transfer success (90%) was higher than the intra-racial crosses. The aedeagus penetrated deep into the bursa, which was slightly stretched as a consequence. This stretching added to the corresponding curvature of the genitalia. Deep penetration of the aedeagus was probably due to the relatively large size of the humphreysii male. Larger males may have had muscular or mechanical advantage over females in this cross that allowed deep penetration and successful spermatophore transfer, despite the short length of the aedeagus and mismatched genital morphologies. In particular, the proximity of the aedeagus to the anterior spermathecal opening may have offset the consequences of mismatched genitalia predicted by Cohn and Cantrall (1974).

In the inter-racial cross, B. h. cochisei male X B. h. humphreysii female, the aedeagus only shallowly penetrated the bursa. Shallow penetration was apparently a direct consequence of relatively small male body size in this cross. Inadequate penetration may have increased the distance between the aedeagal and spermathecal openings, worsened the fit of the mismatched genitalia (Cohn and Cantrall, 1974), and resulted in a very low spermatophore transfer rate (58%) compared to the other crosses.

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THE LOCK-AND-KEY HYPOTHESIS: A HISTORICAL PERSPECTIVE AND RE-EVALUATION

Dufour's lock-and-key hypothesis of mechanical isolation.

Morphologists and systematists have long been intrigued by the diversity and complexity of insect genitalia, recognizing that closely related species can often be distinguished solely on the basis of their reproductive morphologies. As early as 1844, in a pre-Darwinian attempt to explain reproductive isolation, Dufour suggested the genitalia of some insects may act as a lock-and-key mechanism preventing inter-specific insemination. Dufour's hypothesis states that the male intromittent organ (key) is so specific in structure that it only fits inside a female bursa (lock) of complementary form. Genitalia which fit together in this complementary fashion are considered morphologically compatible, while genitalia showing no correspondence are considered morphologically mismatched.

According to Dufour's hypothesis, compatible genitalia should fit together precisely to allow insemination, but incompatible genitalia should mechanically block sperm transfer. In this manner, functionally incompatible genitalia can maintain reproductive isolation between closely related species. Although Dufour believed mismatched genitalia could act as isolating mechanisms, his conception of incompatibilty was purely descriptive and mechanistic. Dufour's hypothesis suggests nothing of what contemporary biologists now recognize as Darwinian natural selection, nor does it suggest the potential role of natural selection in the evolution of mechanical reproductive isolation.

After Darwin's publication of The Origin of Species, biologists recast Dufour's descriptive lock-and-key analogy in evolutionary terms. The lock-and-key hypothesis came to mean that natural selection should act to reinforce and amplify existing genitalic differences between closely related, interbreeding taxa. According to this contemporary view, selection against mechanical incompatibility at the time of copulation should increase inter-taxon genitalic differences by reproductive character displacement/reinforcement (Brown and Wilson, 1957), and eventually eliminate successful interbreeding. In this manner, genitalic displacement/reinforcement could ultimately establish reproductive isolation and lead to the formation of distinct species (Dobzhansky, 1940, 1970; Eberhard, 1985; Mayr, 1963)

Although the minimum conditions for genitalic displacement/reinforcement have never been explicitly stated in the primary or summary literature of evolutionary biology (e.g. Dobzhansky, 1940, 1970; Eberhard, 1985; Futuyma, 1979; Mayr, 1963; White, 1978), Darwinian natural selection should favor the displacement/reinforcement of genitalia under the following conditions:

(1) If closely-related taxa are in geographic contact and are interbreeding. [Primary and secondary contact may be indistinguishable (Endler, 1977).]

(2) If random mating exists within and among the taxa. (Random mating is a minimum requirement. In this case, negative assortative mating would oppose selection for mechanical isolation, while positive assortative mating would act in the same direction.)

(3) If the hybrid offspring of inter-taxon parents are less viable than the offspring of intra-taxon parents (Dobzhansky, 1940,

1970). [Viability differences can contribute to differences in overall fitness (Endler, 1986).]

(4) If inter-taxon differences in genitalia are sufficiently heritable. (Sympatric versus allopatric origins of these character differences may be indistinguishable.)

(5) If genitalic differences impair inter-taxon sperm transfer compared to intra-taxon sperm transfer. (Mechanical incompatibility will translate into fitness differences at the time of copulation.)

Under these conditions, selection should favor intra-taxon matings, but act against inter-taxon matings in areas of geographic contact. Mechanical inefficiencies associated with mismatched genitalia should be common among inter-taxon matings, rendering these crosses less capable of insemination than the intra-taxon crosses. Intra-taxon matings should have a higher proportion of morphologically compatible matings, and will be favored over inter-taxon matings at the time of copulation. If genital morphologies are sufficiently heritable, and provided other selective forces do not oppose selection for intra-taxon matings (Sober, 1984), then the higher insemination success of intra-taxon matings should translate into greater numbers of offspring having intra-taxon (as opposed to hybrid) morphologies. This differential insemination should reinforce genitalic divergence initiated in previous, pre-contact generations.

In addition, viability selection against hybrid offspring should eliminate hybrid-intermediate and backcross genital morphologies. Only genital morphologies associated with the progeny of compatible matings (primarily from intra-taxon parents) will be favored. Reversion of the taxa to similar genital morphologies is unlikely as long as hybrids

remain relatively unfit. In this manner, viability selection against hybrid individuals, coupled with selection in favor of morphologically compatible intra-taxon matings, should displace/reinforce existing genitalic differences and promote mechanical isolation and speciation.

Evidence for and against mechanical isolation.

According to Eberhard (1985), much of the evidence for or against the lock-and-key hypothesis is weak. The following is a synopsis of his extensive review of the literature.

(1) Documenting sperm transfer. Documented cases of genitalic (usually inter-specifc) mismatings are often cited as evidence against the lock-and-key hypothesis. But many studies of inter-specific matings fail to demonstrate the transfer of sperm to the female. If reduced or disrupted sperm transfer lowers the reproductive fitness of mismatched copulating pairs, then the lock-and-key hypothesis remains a viable explanation of mating success.

(2) Female receptivity. Many studies demonstrate inter-specific, morphologically incompatible matings, but do not consider alterations in female receptivity. If females capable of multiple-matings are less willing to remate following an inter-specific or genitalically incompatible encounter, they will be selected against over the course of their reproductive lifetimes. Again, the lock-and-key explanation cannot be rejected.

(3) Mating behavior and genitalic complexity. A lack of pre-copulatory courtship differences between species with divergent genitalia is often cited as evidence for mechanical isolation

(Rentz, 1972; Eberhard, 1985). Presumably, strongly divergent genitalia result from natural selection for mechanical isolation because premating behavior has failed to prevent inter-specific matings. Yet, Eberhard has shown that the correspondence between genitalic differences and a lack of premating behavior does not hold in all cases (Eberhard's Table 2.2). He notes that the precise behavioral cues associated with mate recognition are often difficult to determine, and argues that inconsistencies in the correlational data should therefore be expected. Eberhard finds that the sexual selection of genitalic morphology explains the association between elaborate genitalia and the lack of premating behavior (discussed later).

(4) Character displacement and related predictions. If genitalic morphologies between closely related taxa are similar in allopatry, but suddenly diverge over short geographic distances when taxa are in sympatric contact, this is often taken as evidence of mechanical isolation by genitalic character displacement/reinforcement (e.g. Barytettix psolus and B. paloviridis; Cohn and Cantrall, 1974). But Eberhard (1985) and Grant (1975) argue that character displacement is very difficult to prove. Consequently, Eberhard tested a prediction of the lock-and-key hypothesis indirectly related to character displacement using data from the literature. Specifically, he noted that species continuously isolated from their nearest relative since the original speciation event should not show the same degree of genitalic differentiation as species periodically coming into sympatric contact and experiencing selection for genitalic character displacement. In testing

> this prediction with species on islands, and with parasitic species (a host is a kind of island), Eberhard found no general agreement with the lock-and-key hypothesis. Many island species widely separated from near relatives, and with little opportunity to interbreed, possess genitalia as distinctive as the genitalia of species having ample opportunities to interbreed. Similarly, closely related parasites with no known relatives on the same host (i.e. no chance for interbreeding) have genitalia as distinctive as the genitalia of related parasites that share common hosts.

Eberhard's survey of the literature of mechanical isolation is exhaustive, drawing attention to the lack of critical tests of the lock-and-key hypothesis. He points out that reproductive fitness arguments associated with the lock-and-key remain speculative because the data of sperm transfer and female receptivity are absent. Additionally, he shows that studies correlating genitalic morphology with a lack of premating behavior are ambiguous. Finally, Eberhard suggests most of the evidence for genitalic character displacement used to support the lock-and-key hypothesis can be dismissed due to a lack of rigor and demonstrability.

Eberhard has employed an ingenious biogeographic hypothesis for testing mechanical isolation that appears to avoid the difficulties of documenting genitalic character displacement. However, I believe all he can reasonably argue from his data is that distinctive, species-specific genitalia can evolve both in total allopatry, and perhaps through periodic inter-taxon contact. Certainly distinctive genitalia are found among species in both of these geographic classes. Furthermore, we never know if, or for how long, his potentially interbreeding taxa were

in contact such that genitalic character displacement could evolve through natural selection. Therefore, there is no real basis for evaluating the lock-and-key hypothesis in the context of natural selection, a condition Eberhard rightfully insists is important. Like Grant (1975), I believe the best studies of character displacement/reinforcement must take place within and adjacent to zones of inter-taxon contact. Such studies, for the most part, are absent from Eberhard's data set.

Alexander's perspective: the inefficiency hypothesis.

Alexander (1962, 1964) considers genitalic incompatibility and mechanical isolation inefficient barriers to hybridization. Even though mechanical isolation may complelety prohibit the formation of hybrid progeny, it requires copulation to be effective. Thus, gametes and energy are wasted on a mechanically impossible mating, and the copulating pair is unnecessarily exposed to predation. Males and females who recognize and mate correctly with their own type will be less susceptible to predation, and more likely to invest a presumably limited supply of gametes in mechanically feasible matings. Alexander argues that the reduction in fitness associated with a total reliance on genitalic barriers to reproduction may prohibit the evolution of mechanical isolation. Instead, he believes divergent courtship behaviors should evolve as isolating mechanisms because they avoid the lowered fitness associated with mis-copulation. According to Alexander, genitalic morphology should diverge strictly as a by-product of behavioral isolation (Muller, 1940; Mayr, 1963), not through the direct selection of genitalic incompatibility as a barrier to hybridization as the lock-and-key hypothesis suggests.

I believe Alexander's arguments are sound provided there is concurrent selection on both genitalic and behavioral isolating mechanisms. In other words, if selection is operating on genitalic compatibility and premating behaviors at the same time, and if selection optimizes energetic or gametic expenditures, then premating isolating barriers should be favored over genitalic barriers.

By Alexander's reasoning, genitalic incompatibility is less efficient than premating behavior as an isolating mechanism; therefore, selection on genitalia should cease in favor of selection on premating behavior. However, if genitalic differences affecting insemination are present among closely related taxa, but premating behaviors are virtually absent, then it seems more likely genitalic barriers will develop before behavioral ones. Alternatively, if positive assortative mating accompanies mechanical incompatibility, or if the fit of the genitalia is used as a basis for mate choice, then mating behaviors and genitalic differences may co-evolve as a common isolating mechanisms. Eberhard presents such a view of genitalic evolution in his sexual selection hypothesis.

Eberhard and the sexual selection hypothesis.

Eberhard (1985) suggests genitalic divergence is neither a by-product nor a direct consequence of natural selection for reproductive isolation. (The former is Alexander's argument, the latter is the lock-and-key hypothesis.) Instead, he believes genitalic evolution results from the sexual selection of male gentalia by active female choice. Eberhard proposes that male genitalia can function as "internal courtship" devices which influence the probability a female will use a given male's sperm. Mechanically inferior males, or those unrecognizable

by tactile stimulation, will be behaviorally rejected by females in the early stages of copulation. Thus, given sufficient genetic variation in the female discrimination of male genitalia, runaway sexual selection (Fisher, 1958; Lande, 1981) may cause the rapid divergence of male genitalia.

Eberhard believes that sexual selection by female choice may explain the greater taxonomic diversity of male genitalia compared to female genitalia. According to his reasoning, if male genitalia serve as tactile signals of mate identity or of the ability to transfer sperm, then male morphologies will likely be elaborated by sexual selection through the discriminating choice of females. But if females rely on the tactile stimulation of copulation for discriminating among males, then female neurological/behavioral features will be more likely to evolve by sexual selection than genital morphologies. In contrast, the lock-and-key hypothesis predicts equal diversification of the male and female genitalia. The lock-and-key hypothesis assumes a basic complementarity between the male and female genitalia such that both sexes must co-evolve to maintain the biological function of sperm transfer. The genitalia of each sex are functionally constrained so that neither sex can change without triggering selection for a complementary change in the opposite sex.

Eberhard also notes that sexual selection should arbitrarily elaborate male morphologies, regardless of their mechanical importance to sperm transfer (see also Brown, 1975; Fisher, 1958). In support of this point, Eberhard cites many studies suggesting no clear mechanical function for the highly elaborated or complicated genitalic components under investigation. This lack of functional significance seems particularly damning to the lock-and-key hypothesis because genitalic

divergence and reproductive isolation are assumed to directly result from selection for mechanical/functional compatibility.

Under the weight of an extensive literature review, Eberhard has dismissed the lock-and-key hypothesis as a viable explanation for the origin and maintenance of genitalic diversity, suggesting the evidence for mechanical isolation is weak and inconclusive. Eberhard believes the hypothesis of sexual selection by female choice better explains the rapid divergence of male genitalia. With sweeping clarity, sexual selection can explain the rampant diversity and rapid evolution of genitalia in nature. Not only does sexual selection account for the greater elaboration of male genitalia compared to female genitalia, but it also explains the lack of premating behavior among species having complicated genitalia. According to Eberhard, if elaborate genitalia characterize a species, then courtship behaviors most likely concern the recognition of genitalic stimulation, not premating displays.

If Eberhard's sexual selection hypothesis is correct, the evolution of genitalic morhphology may have less to do with the mechanics of sperm transfer that is so important to the lock-and-key hypothesis, and more to do with the quantitative components of a sexually selected system: a large genetic covariation between female mating preferences and the sexually selected male genitalia, weak natural selection on the male genitalia, and stereotypic female preferences for the tactile or mechanical properities of the male genitalia (after Arnold, 1985). But as noted below, the study of sexual selection has its own difficulties.

Despite the appeal of Eberhard's arguments, sexual selection explanations have problems as great as the difficulties encountered with the lock-and-key hypothesis. For example, in order to imply that the evolution of mechanical isolation by natural selection is taking place,

it is first necessary to demonstrate the presence of additive genetic variation in genital morphology, as well as differences in sperm transfer (reproductive fitness) that are associated with genitalic incompatibility. But to infer that evolution by sexual selection is operating, not only must genetic variation in male genital morphology be demonstrated, but genetic variation in female mate choice must also be substantiated. Once these critical parameters have been established, it still remains to be demonstrated that genetic covariation between male morphology and female choice is sufficient to overcome natural selection on the male genitalia to the extent that runaway sexual selection will ensue (Lande, 1981).

In addition to documenting the necessary genetic parameters, differences in reproductive fitness due to mismating may not be as easily verified with sexual selection models as with the lock-and-key hypothesis. According to the lock-and-key hypothesis, reproductive fitness is narrowly defined as the functional/morphological ability to transfer sperm from the male to the female, and can be readily measured as the frequency of successful insemination. But with sexual selection, the male genitalia may advertise one or more components of reproductive fitness that are not immediately apparent to the investigator (e.g. mechanical compatibility, mate identity, viability, genetic quality, mating vigor, etc.). As a null hypothesis, it may be much easier to test and discount the lock-and-key hypothesis than the sexual selection hypothesis.

The utility of the lock-and-key analogy.

Many investigators recording mismatched or inter-specific copulations have concluded that genitalic incompatibility is not a viable evolutionary mechanism leading to reproductive isolation (Eberhard, 1985). Often in these studies, a failure to observe inter-specific genitalic differences that inhibit copulation is grounds for dismissing the lock-and-key hypothesis. Some investigators have suggested that any inter-taxon transfer of sperm allowed by incomplete mechanical barriers is selectively disadvantageous. Consequently, mechanical isolation should be selected against in favor of premating isolation (Alexander, 1964). But such a focus overlooks the important population-level manifestations of genitalic incompatibility during intermediate stages of evolution. If selection for mechanical isolation is ongoing, and mechanical barriers are incomplete, then insemination success should be strongly associated with the various male and female genital morphologies in the population. Under the lock-and-key hypothesis, the distribution of male and female morphologies determines the type and frequency of genitalic matings that are possible (given random mating) and determines the mechanical efficiencies associated with each. Slight changes in the morphological composition of the population may place new constraints on the types of genitalic fit that are possible. If these changes alter the frequency of insemination sufficiently, selection may favor the most advantageous morphologies in the population (a point alluded to by M. J. D. White in Alexander, 1964). Investigators expecting genitalic differences between related taxa to strictly correspond to total mechanical isolation run the risk of refuting the lock-and-key hypothesis precisely in those cases where genitalic incompatibility is currently under selection.

The lock-and-key hypothesis of Dufour implies that a morphologically precise and complementary fit of male and female structures is required for insemination to occur. But as noted above, this formulation places unrealistic expectations on the outcome of genitalic incompatibility during critical intermediate stages of evolution. Furthermore, a perfect correspondence in male and female genital structures is unknown in nature (Eberhard, 1985). Every bump and prominence on the surface of the male intromittment organ is not matched by a corresponding concavity in the female bursa, and the dimensions of male and female genitalia seldom precisely correspond. This is a predictable outcome of naturally occuring genetic variation among individuals in a population and the confounding influence of environmental variation on genetically heritable traits.

In natural populations, variation in complex genotypic and phenotypic characters is common. Given the magnitude of variation associated with most morphological traits, it is unlikely the perfectly-fitting genitalic mate for any single individual will be encountered, or if such a mate even exists. Thus, it should not be too surprising if variation in genitalic fit prevails over precisely corresponding fits in natural populations. [Furthermore, to maintain a perfect lock-and-key fit on an evolutionary timescale, inter-sexual correlations in genitalic morphologies must be high from generation to generation. Since sex-influenced and/or and sex-linked inheritance is undoubtedly associated with the genitalic morphology, and if this type of inheritance can disrupt genotypic and phenotypic character correlations (see Arnold, 1985); it seems unlikely a perfect lock-and-key fit could be maintained for more than a few generations before inter-sexual character correlations were broken down and variation in genitalic fit restored.]

Phenotypic variation in morphology is the dominant theme in nature, and heritable variation is also critical to morphological evolution by natural selection (Endler, 1986). Consequently, tests of the lock-and-key hypothesis should unambiguously assess the quantitative effects of genitalic variation on reproductive fitness. Specifically, tests of the lock-and-key hypothesis should relate quantitative variation in the fit of the genitalia to differences in insemination success. When recast in this fashion, the lock-and-key hypothesis predicts that greater variation in genitalic fit is associated with lower frequencies of insemination, and less variation in genitalic fit is associated with higher frequencies of insemination. In this manner, the lock-and-key hypothesis is more properly aligned with methodologies useful in testing adaptive morphologies at the population level, and can be clearly interpreted in the context of selection theory (Endler, 1986).

Measuring genitalic incompatibility: the central problem.

Increasing attention is being focused on the inadequacy of the lock-and-key hypothesis in explaining genitalic diversity (summarized by Rentz, 1972, and Eberhard, 1985). Yet as pointed out by Eberhard, much of this work remains problematic and controversial. As seen in the foregoing discussion, a major source of uncertainty is not the theoretical basis of the work, but a misunderstanding of the population-level consequences of genitalic incompatibility. Since variable morphological fit of the genitalia is presumably common within a population, and also associated with alterations in insemination frequencies, variation in genitalic fit must be quantified among suitably large samples of copulating individuals to adequately test the lock-and-key hypothesis. Virtually all studies of the lock-and-key

hypothesis lack precise estimates of how well the genitalia fit together and fail to address the effects of variation in genitalic fit on insemination success.

Quantification of variation in genitalic fit is also desirable if research on genitalic incompatibility is to be incorporated into the greater body of quantitative selection theory (e.g. Arnold and Wade, 1984a, 1984b; Lande and Arnold, 1983). Preferably, estimates of genitalic fit should be accompanied by measures of genitalic morphology among copulating and non-copulating individuals in natural populations. In addition, measures of the amount of sperm transferred, or at least an indication of sperm in the female after copulation, are necessary to calibrate the fit of the genitalia with a related component of reproductive success. These are the critical data required to test mechanical isolation.

The lock-and-key hypothesis clearly relates variation in the genitalia to variation in the morphological fit of the genitalia during copulation. In turn, variation in genitalic fit may mechanically influence insemination success and alter reproductive fitness relationships between interbreeding taxa. Without testing this causal relationship between genitalic form, genitalic function, and mating fitness, there can be no conclusive demonstration of natural selection for mechanical isolation (after Arnold, 1983). Furthermore, without meaningful quantitative estimates of genitalic fit and insemination success, it may be difficult to distinguish among the the lock-and-key hypothesis and its competing theories.

Summary of chapter one

Dufour's lock-and-key hypothesis, as understood by contemporary evolutionary biologists, is only one of several attempts to account for the evolution of genitalic diversity as a consequence of selection. The lock-and-key hypothesis predicts that genitalic morphologies evolve as a direct consequence of natural selection for mechanical reproductive isolation during speciation. Genitalic incompatibility and hybrid unfitness are important assumptions of this hypothesis. In contrast, Alexander believes genitalic isolating mechanisms lower reproductive fitness by wasting gametes on mechanically impossible matings. He expects genitalic barriers to be rare and quickly replaced by behavioral courtship differences. According to Alexander's view, genitalic divergence should be a by-product of speciation, not a direct consequence of natural selection for reproductive isolation. Eberhard disagrees with both of these hypotheses. Instead, he believes the genitalia serve as internal courtship devices that influence the probability that a female will utilize a given male's sperm. According to Eberhard, genitalic divergence is a consequence of sexual selection by female choice, and may be neither a by-product, nor a direct consequence of natural selection for reproductive isolation and speciation.

The lock-and-key analogy was seen to have limited utility in conceptualizing the population-level manifestations of genitalic incompatibility. Variation in genitalic morphology is probably common among natural populations, and therefore variation in genitalic fit is also expected. Investigators searching for complete mechanical isolation when differences in genital morphology are known, may miss variation in genitalic fit that is currently under selection during

intermediate stages of reproductive isolation. As a criterion for testing the lock-and-key hypothesis, expectations that mismatched genitalia will perfectly correspond with complete mechanical isolation may lead investigators to reject the lock-and-key hypothesis, when in fact it is true. A more powerful and realistic test of the lock-and-key hypothesis would predict that variation in genitalic fit is statistically associated with changes in insemination success in a large sample of copulating pairs.

CHAPTER 2

THE LOCK-AND-KEY HYPOTHESIS IN BARYTETTIX

Barytettix grasshoppers as a study group.

Evaluating the operation of genitalic incompatibility requires taxa currently or recently under selection for mechanical reproductive isolation. Characterstics of a tractable study system should include few premating, non-morphological barriers to breeding and an occasional tendency for taxa to cross. Grasshoppers of the melanopline genus Barytettix form a group of nine species that appear to meet these criteria (Cohn and Cantrall, 1974). Adult males initiate sexual activity by leaping upon females and immediately attempting to copulate (figure 1). Very little, if any courtship behavior appears to be involved (Cohn and Bennack, unpublished observations). In addition, inter-specific and inter-racial matings are known in the field and lab (Cohn and Cantrall, 1974; Bennack, this study).

According to Cohn and Cantrall, the only reproductive barriers between Barytettix species in zones of contact may be genitalic differences. [Major anatomical features of the genitalia (the male aedeagus and the female bursa) are illustrated in figures 2 and 3.] B. psolus and B. paloviridis exhibit genitalic character displacement in sympatry in western Mexico, but do not hybridize naturally. B. humphreysii humphreysii and B. h. cohcisei have distinct genital morphologies in allopatry, hybridize readily in the lab and field (Cohn and Cantrall, 1974; Cohn, unpublished data), and show graded changes in genital morphology across several ecotonal hybrid zones in Arizona and northern Mexico. Electrophoretic data also indicate B. h. humphreysii

Figure 1. Copulating pair of Barytettix grasshoppers. The male mounts from above and twists his abdomen under the female to insert the intromittent organ (aedeagus). The mechanical consequence of this twisting is that dorsal aedegal structures are rotated 180 degrees to become inserted ventrally into the bursa.

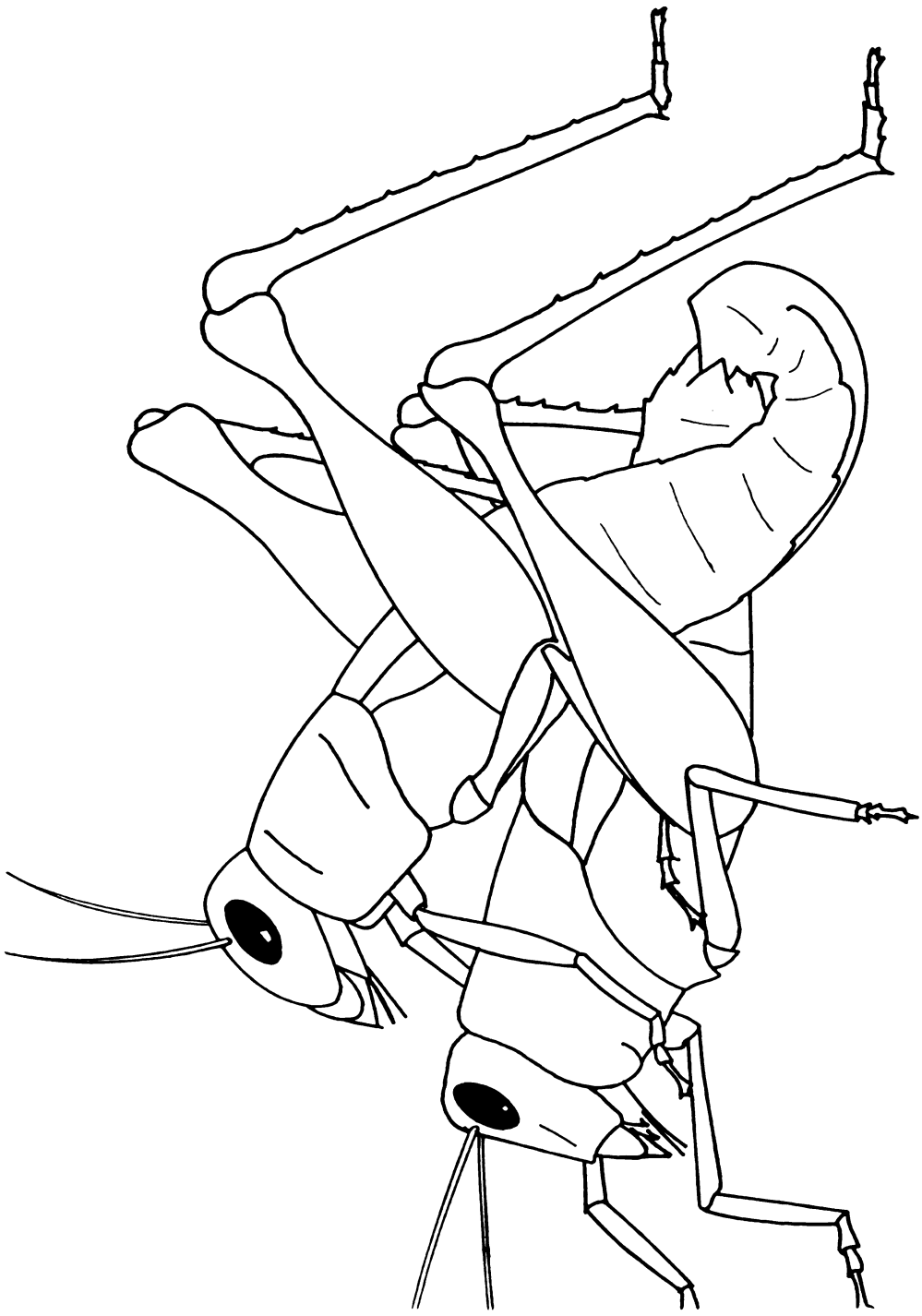


Figure 2. Barytettix paloviridis, semi-diagrammatic view of the male genitalia (from Cohn and Cantrall, 1974).

A. lateral view

B. ventral view

Symbols used in figure:

DV	-dorsal valve
PRC	-process of ramus of cingulum
RC	-ramus of cingulum
SVL	-sclerite of ventral lobe of ectophallic membrane
VC	-ventral cleft
VLRC	-ventral lobe of ramus of cingulum
VLSH	-ventral lobe of sheath
VV	-ventral valve

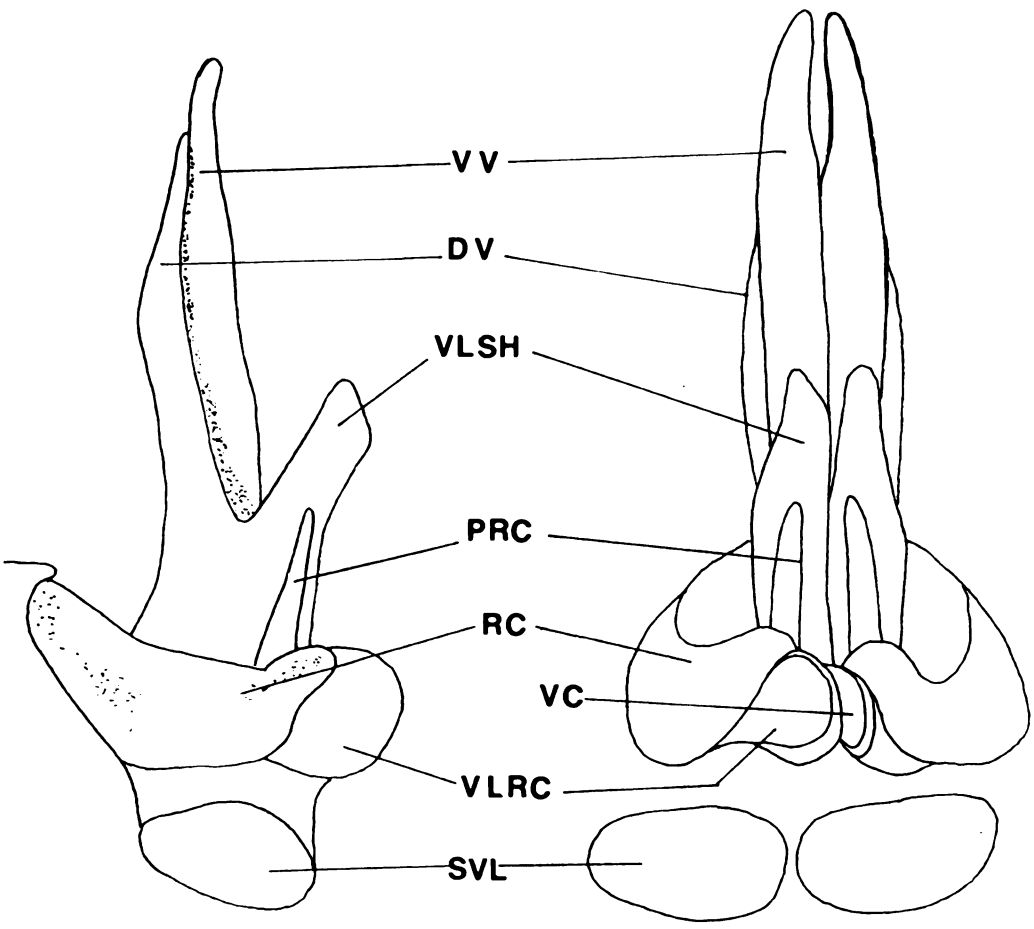
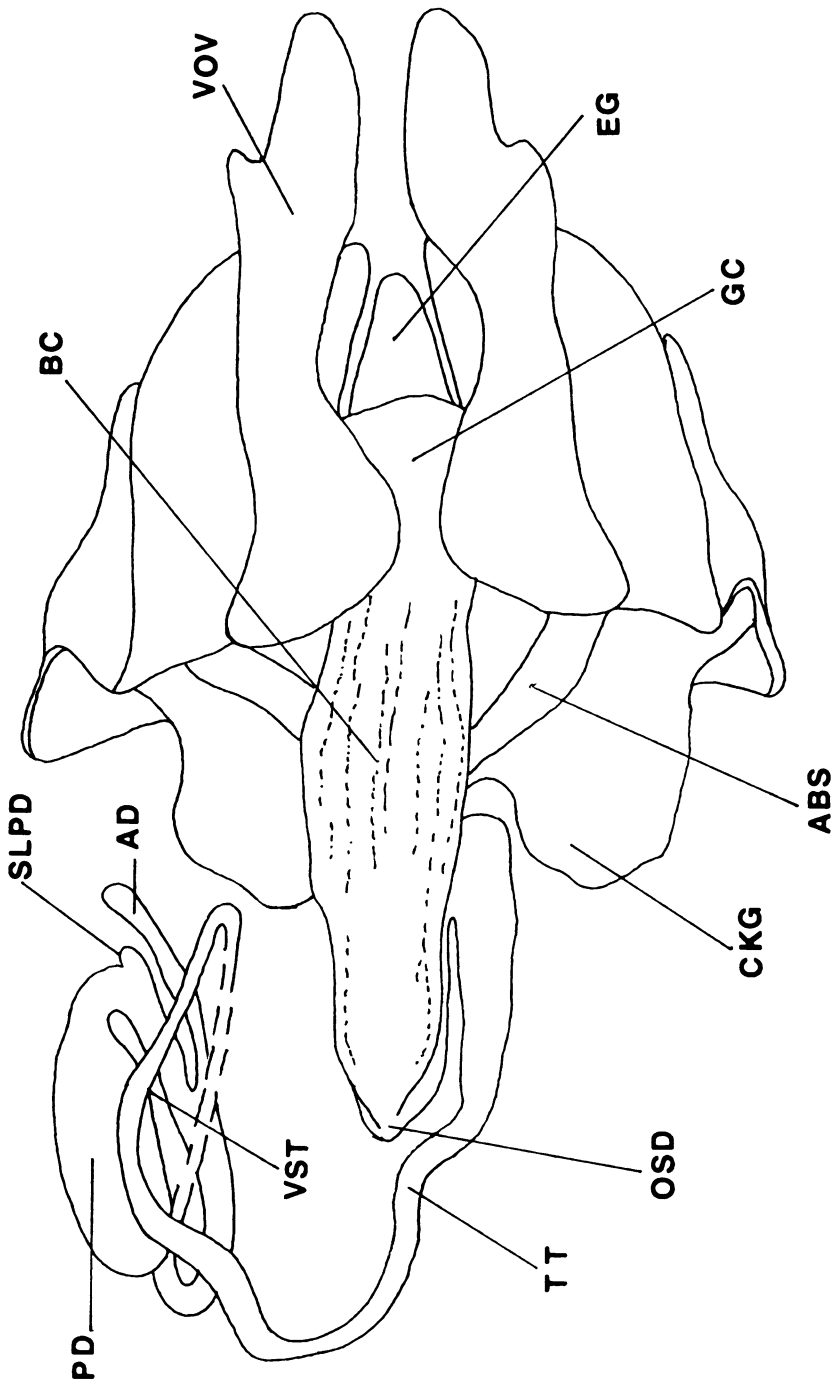

VV
DV
VLSH
PRC
RC
VC
VLRC
SVL
A
B

Figure 3. Barytettix paloviridis, female internal copulatory elements (from Cohn and Cantrall, 1974).

Symbols used in figure:

AB -anterior basivalvular sclerite
AD -apical diverticulum of spermatheca
BC -bursa copulatrix
CKG -Comstock-Kellogg gland
EG -egg guide
GC -genital chamber
OSD -opening of spermathecal duct
PD -preapical diverticulum of spermatheca
SLPD -secondary lobe of preapical diverticulum
TT -thin tube of spermathecal duct
VOV -ventral ovipositor valves
VST -vestibule


VOV
EG
GC
BC
ABS
CKG
SLPD
AD
VST
OSD
TT
PD

and B. h. cochisei are incompletely isolated (Bennack and Howard, unpublished data). In a recent pilot study, Bennack and Howard found these races differentiable at only one of 18 allozyme loci where each racial population was fixed for a differenent allele. Cohn and Cantrall have concluded that mechanical isolation may be virtually complete in B. psolus and B. paloviridis but only weakly developed in B. h. humphreysii and B. h. cohcisei. The electrophoretic data of Bennack and Howard confirm incomplete isolation in the latter case.

The advantages of B. humphreysii humphreysii and B. h. cochisei.

I chose to quantify and study the effects of genitalic incompatibility on insemination success in hybridizing races of B. humphreysii in southeastern Arizona. These large, flightless grasshoppers are easy to observe, readily acessible within U. S. territorial borders, and among the most common grasshoppers within their range. Racial differences in genitalic morphology are pronounced (figure 4). Furthermore, Cohn and Cantrall (1974) believe genitalic morphology strongly influences the ability of these races to hybridize (discussed below).

B. h. humphreysii and B. h. cohcisei are particularly wellsuited to studies of mechanical incompatibility because they engage in lengthy, quiescent copulations during late afternoon and well into the evening. At these times, cooler temperatures slow motor reflexes, making them extremely easy to approach without disturbing copulation. These grasshoppers are also easy to breed, readily mate in the laboratory, and can be reared from hatching to maturity on Romaine lettuce and supplemental local vegetation (Cohn and Bennack, unpublished observations).

Figure 4. Genital morphology in Barytettix humphreysii humphreysii and B. h. cochisei.

A. Schematic representation of the aedeagus and bursa during copulation showing the anatomical relationships common to both B. h. humphreysii and B. h. cochisei. Note that male structures are rotated 180 degrees as explained in figure 1. Oblique view.

B. Ventral aedeagal valves (ventral view) and bursa (dorsal view) in B. h. cochisei.

C. Ventral aedeagal valves and bursa in B. h. humphreysii. (Same views as in B.)

Aedeagal valves and bursae in B and C are drawn to the same scale. Dorsal aedeagal valves are very similar in the two races and thus are not illustrated. Anterior basivalvular sclerite is included as a reference structure.

Symbols used in the figure:

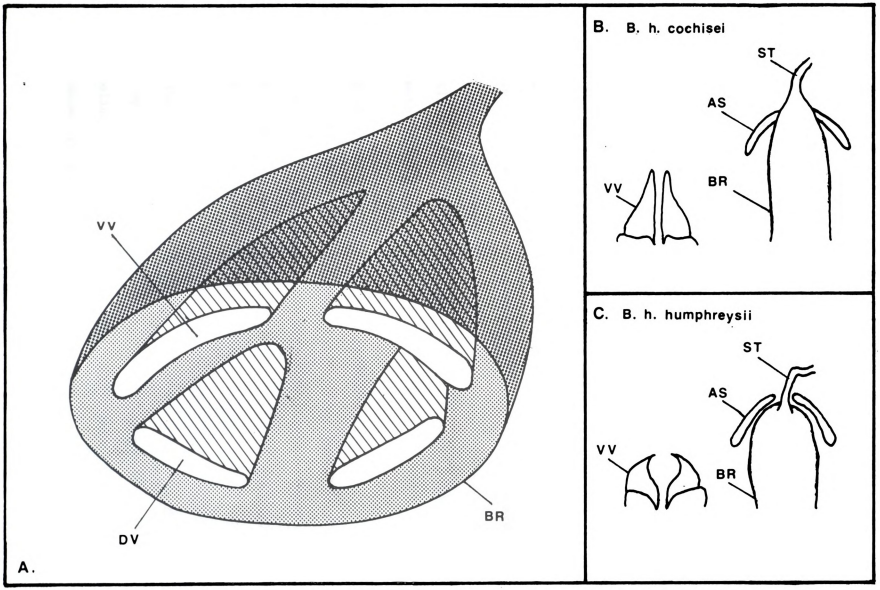
AS -anterior basivalvular scelerite

BR -bursa

DV -dorsal valve

ST -spermathecal tube

VV -ventral valve


A.
VV
DV
BR
B. B. h. cochisei
ST
AS
VV
BR
C. B. h. humphreysii
ST
AS
VV
BR

The ability of these races to hybridize is particularly desirable. A stronger test of the effects of variation in genitalic fit can be made in incompletely isolated races than in fully isolated species. Fully mechanically isolated species should be incapable of cross-insemination, regardless of the level of variation in genitalic fit. Without some degree of insemination, there is no standard by which to judge the effects of variable genitalic fits. The only experimental recourse is the surgical alteration of the genitalia to create artifical variation, but there is no guarantee surgical effects on insemination success are not artifactual. However, if a lock-and-key mechanism is currently under selection in incompletely isolated races, then changes in insemination success should strongly correspond with variation in genitalic fit, both in intra-racial and inter-racial crosses. This simple assumption allows the use of conventional regression techniques to statistically test the lock-and-key hypothesis under controlled experimental conditions.

The mode of insemination in Barytettix grasshoppers.

In most grasshopper species, males signal females in a species-specific fashion in an effort to initiate copulation. However, in the sub-family Melanoplinae, which includes the genus Barytettix, male behavior is strikingly different. Males intiate sexual activity by leaping upon the female and immediately attempting to copulate (Otte, 1970, 1981). Only after mounting the female does the male signal, usually by shaking his hind legs in a species-characteristic fashion.

Preliminary observations of B. h. humphreysii and B. h. cohcisei males show no readily observable differences in leg-shaking behavior that may influence the outcome of copulation (Cohn and Bennack,

unpublished observations). Instead, differential inter-racial insemination is expected to result from misplacement of the male spermatophore tube within the female bursa due to genitalic incompatibility (Cohn and Cantrall, 1974).

Successful insemination in Barytettix and many other grasshoppers depends on the eversion of a male spermatophore tube into the spermathecal duct of the female, followed by the passage of sperm through the tube and into the spermathecal duct and spermatheca (Bennack, unpublished data; Ewen and Pickford, 1975; Gregory, 1965; Hartmann, 1970; Pickford and Gillot, 1971; Pickford and Padgham, 1973). Cohn and Cantrall suggest that the penetration of the spermatophore tube in Barytettix could depend upon the precise fit of the male intromittent aedeagus inside the female bursa (figure 4a).

At the population level, unacceptable variation in the fit of the genitalia could prevent consistent alignment of the aedeagal orifice with the opening of the female spermathecal duct, and lead to a reduction in insemination success. Conversely, invariant fit along critical regions of the coupled genitalia may serve to stabilize the copulatory structures and increase the frequency of successful insemination.

The predicted fate of intra-racial and inter-racial copulations.

In most copulating acridid grasshoppers, including Barytettix, the male mounts from above and twists his abdomen under the female to insert the aedeagus (figure 1). The mechanical consequence of this twisting is that dorsal aedeagal structures are rotated 180 degrees to be inserted ventrally into the bursa (figure 4a). Cohn and Cantrall (1974) believe successful transfer of the spermatophore tube is a function of the

alignment and morphological complementarity of the male and female genitalia after this 180 degree rotation. Based on their knowledge of genital morphologies in Barytettix, Cohn and Cantrall offered the following predictions on the fate of the spermatophore tube in intra-racial and inter-racial crosses of B. h. humphreysii and B. h. cochisei.

In B. h. cochisei males, the ventral aedeagal valves are elongate (figure 4b), and their medial edges presumably close enough together to cause the spermatophore tube to issue distally from the aedeagus toward the anteriorly located spermathecal duct in B. h. cochisei females (figure 5a). But in B. h. humphreysii, the inner margins of the ventral valves are markedly concave, leaving a gap in the center of the ventral valve complex (figure 4c). According to Cohn and Cantrall, this gap would allow the underlying dorsal valves to successfully turn the spermatophore toward the antero-dorsal opening of the spermathecal duct in B. h. humphreysii females (figure 5b). Cohn and Cantrall believe these particular intra-racial morphologies of each sex are functionally compatible, and will enhance spermatophore transfer and insemination in intra-racial crosses.

Cohn and Cantrall predict a very different outcome in inter-racial matings. The ventral valves of B. h. cochisei are expected to cover the antero-dorsal orifice of the spermathecal duct of B. h. humphreysii, to turn the spermatophore tube toward the anterior bursal wall, and thus to prevent insemination (figure 5c). In B. h. humphreysii, the dorsal valves should turn the spermatophore upward through the ventral valve gap and into the dorsal wall of the bursa, rather than guide it to the anteriorly located spermathecal duct of B. h. cochisei (figure 5d). In both cases, males of one race should be mechanically unable to inseminate females of the other race.

Figure 5. Schematic representation of the fate of the spermatophore tube in crosses of Barytettix humphreysii races. In the HMCF cross, mismatched genital morphologies may be offset by a deep penetration of the aedeagus that results from a relatively large male body size. The fit of the mismatched genitalia in the CMHF cross may have been worsened by a shallow aedeagal penetration resulting from a relatively small male size. (The distance between the spermathecal duct opening and the apex of the aedeagus is drawn approximately to scale. Black arrows represent the probable path of the spermatophore tube. One millimeter equals 10 microns.)

A. Predicted CMCF cross (B. h. cochisei male X B. h. cochisei female). The distance between the apex of each valve and the opening of the spermathecal duct is proportional to the average distance observed in this experimental cross.

B. Predicted HMHF cross (B. h. humphreysii male X B. h. humphreysii female). The distance between the apex of each valve and the opening of the spermathecal duct is proportional to the average distance observed in this experimental cross.

C. Predicted CMHF cross (B. h. cochisei male X B. h. humphreysii female). Compared to the humphreysii male, the relatively longer ventral valves of the cochisei male (medial margins of the valves close together) should block the spermatophore tube from the dorsal opening of the humphreysii spermathecal duct.

D. Predicted HMCF cross (B. h. humphreysii male X B. h. cochisei female). Compared to the cochisei male, the relatively shorter ventral valves of the humphreysii male (medial margins of the valves form a gap) should direct the spermatophore tube dorsally, away from the anterior opening of the cochisei spermathecal duct.

E. Observed HMCF cross. The aedeagus penetrated more deeply than anticipated by Cohn and Cantrall, probably due to the relatively large body size of the humphreysii male (see chapter 4). The distance between the apex of each valve and the opening of the spermathecal duct is proportional to the average distance observed in this experimental cross.

F. Observed CMHF cross. The aedeagus penetrated more shallowly than anticipated by Cohn and Cantrall, probably due to the relatively small body size of the cochisei male (see chapter 4). The distance between the apex of each valve and the opening of the spermathecal duct is proportional to the average distance observed in this experimental cross.

Summary of chapter two and statement of research objectives.

B. h. humphreysii and B. h. cochisei seem well-suited for studies of genitalic incompatibility and mechanical isolation. They possess distinctly different genitalia, are common where they occur, are large and flightless, and easily captured in copula. Furthermore, they are easily maintained in the lab, and will readily cross-copulate. Also, a detailed literature on sperm transfer has allowed Cohn and Cantrall to make predictions about the outcome of copulation in intra-racial and inter-racial crosses of these grasshoppers. Their predictions are an integral part of testing the lock-and-key hypothesis in Barytettix.

At this point, it is important to clearly state that this dissertation work is not a direct test of selection for mechanical isolation between B. h. humphreysii and B. h. cochisei in nature. As mentioned in chapter one, genital morphologies must be measured in copulating and non-copulating individuals to detect the presence of selection in the wild (Arnold and Wade, 1984a, 1984b; Endler, 1986). Instead, this study bears directly on the functional basis of selection for mechanical isolation by testing a specific assumption of the lock-and-key hypothesis: that variation in the fit of the genitalia during copulation is significantly associated with insemination success.
To this end, the following objectives were targeted.

(1) Methods were developed for preserving and sectioning large samples of Barytettix grasshoppers in copula.

(2) Methods were developed for scoring insemination success in copulating pairs.

(3) Geometric estimates of genitalic fit were operationally defined and systematically measured in cross-sections of the copulating genitalia.

(4) The effect of genitalic fit on insemination success was statistically analyzed.

In addition, reciprocal inter-racial crosses of B. h. humphreysii and B. h. cochisei were compared to the intra-racial crosses to assess:

(5) The effect of natural, non-surgical alterations in the type and range of genitalic fits possible when intra-racial morphological bounds are exceeded.

(6) The effectiveness of genitalic reproductive barriers during intermediate stages of speciation in these Barytettix races.

CHAPTER 3

METHODS FOR TESTING THE LOCK-AND-KEY HYPOTHESIS IN *BARYTETTIX*

Study sites and mating design.

Grasshoppers from allopatric populations of *B. humphreysii humphreysii* and *B. h. cochisei* were obtained from field sites in southeastern Arizona in the late summer of 1981. Over five-hundred *B. h. humphreysii* were collected over a three-day period on the lower northern slopes of the Santa Rita mountains in Madera Canyon, 30 miles south of Tucson. In addition, over five hundred *B. h. cochisei* were collected in a similar period of time at the San Bernadino Ranch headquarters, 20 miles east of Douglas in the extreme southeastern corner of Arizona. The San Bernadino site is a typical Chihuahuan desert scrub habitat with creosote bush (*Larrea* sp.), and mesquite trees (*Acacia* sp.) predominating. The Madera Canyon site is a semi-arid grassland occurring just below a mixed coniferous-deciduous upland slope in the Santa Rita mountains. Mesquite and grama grasses (*Bouteloua* sp.) were common, and Chihuahuan and Sonoran flora were noticeably interspersed. Both sites were moderately grazed by cattle. According to local residents, the seasonal rains were on time at both sites in the summer of 1981, and above average in amount.

All grasshoppers were separated by sex in the field and brought to the Southwestern Research Station (SWRS) of the American Museum of Natural History near Portal, Arizona. Grasshoppers were maintained on a diet of Romaine lettuce, bran, and local vegetation. Feeding and cage maintenance occurred every morning between nine and noon. Preparation for the mating experiments occupied the remainder of the day.

Grasshoppers remained isolated by sex for seven days before the mating experiments began. During this time, final instars moulted to the adult stage, and adult females with previous copulatory experience presumably had time to eliminate or absorb any remaining spermatophores (Ewen and Pickford, 1975; Gregory, 1965; Hartmann, 1970; Pickford and Gillot, 1971; Pickford and Padgham, 1973). No females were observed to lay eggs during this holding period, which probably indicates that the majority of females were without previous copulations. However, to ensure that previous copulations did not affect subsequent receptivity to copulation after the seven-day holding period, a subsample of females inseminated during the mating experiment was allowed to lay eggs. Receptivity to copulation returned in 24 hours or less. Another subsample of males appeared receptive to copulation at all times. Thus, males and females at the end of the seven-day holding period were judged receptive to copulation, and the genital tracts of non-virgin females were considered cleared of any previous spermatophores that might bias scores of spermatophore transfer from the mating experiment.

Following the seven-day holding period, I randomly assigned males and females in pairs to individual cages such that the intra-racial and reciprocal inter-racial crosses were represented in equal numbers. All individuals were weighed to the nearest milligram on a triple-beam balance prior to placement in cages. Twenty pairs per mating cross (four crosses; 80 total pairs) were assigned each afternoon at four o'clock, and observed every 30 minutes until midnight. Pairs found in copula were allowed to copulate for 90 minutes to permit adequate penetration of the spermatophore tube (Ewen and Pickford, 1975; Gregory, 1965; Hartmann, 1970; Pickford and Gillot, 1971; Pickford and Padgham, 1973). The pairs were then chilled in a refrigerator to retard motor

reflexes, quickly frozen in copula in a bath of liquid nitrogen, and transferred to a freezer at zero degrees centigrade for temporary storage. The mating of grasshopper pairs continued in this manner for six days.

Histological Techniques.

The genitalia of copulating males and females were removed from the abdomens (at approximately the eighth abdominal segment) after two hours of freezing at zero degrees centigrade. This allowed the male aedeagus, as well as the female spermatheca and bursa, to remain in coupula. The genitalia were fixed in Bouin's solution (Humason, 1979) overnight and transferred to 70% ethanol for transportation to Michigan State University. Fifty pairs of copulating genitalia per cross were randomly selected to be preserved for analysis (and an additional 100 non-copulating individuals for the development of histological techniques), but some copulating specimens were later sacrificed to refine histological procedures. Ultimately, each intra-racial cross of B. h. humphreysii and B. h. cohcisei was represented by a sample size of 31 pairs of copulating genitalia. The sample for the inter-racial cross of B. h. humphreysii males x B. h. cochisei females had 31 specimen pairs, and the reciprocal sample of B. h. cochisei males x B. h. humphreysii females had 38 specimen pairs.

Ten specimens per day were were prepared for serial sectioning according to the following four-day protocol. On day one, genitalic specimens were stepped down through changes of ethanol at 55%, 40%, 35%, and 15% stages for 30 minutes each, and immersed in distilled water for five minutes. Genitalia were then transferred to 10% aqueous potassium hydroxide at room temperature for 12 to 18 hours to soften the

sclerotized portions of the genitalia for paraffin infiltration.

On day two, specimens were removed from the potassium hydroxide solution and immersed for five minutes in distilled water. The genitalia were stepped up through changes of alcohol at 15%, 35%, 40%, 55%, and 70% concentrations for twenty minutes each. Final dehydration was accomplished in two baths of 95% ethanol for five minutes each, and one bath of 100% ethanol for five minutes. Initially, air pockets were common in areas between male and female structures, so final dehydration steps were carried out under partial vacuum at 20 psi. Specimens were immediately transferred from 100% ethanol into methyl salicylate, evacuated for five minutes at 20 psi, and left to clear for 18 hours. Methyl salicylate was preferred as a clearing agent over xylene because xylene often left the genitalia too brittle for sectioning.

Air trapped in genitalic specimens during tissue preparation often led to inadedquate embedding and could cause entire specimens to be destroyed during sectioning. Consequently, every effort was made to avoid exposing the coupled genitalia to air during fluid changes. To facilitate this process, all specimens were placed in small, individual transfer vials. In these open-ended vials, specimens completely submersed in one fluid could be immersed in a much larger vessel containing the second fluid change. Desired changes in fluid concentration around the specimen occured by diffusion between the larger bath and the vial, through the vial's open end.

On day three, methyl salicylate was removed from the genitalia and replaced by Paraplast, a paraffin/plastic infiltrating and embedding medium. To accomplish this replacement of the clearing agent, the genitalia were stepped through two mixtures of methyl salicylate and Paraplast for 15 minutes each. The first change was a 75:25 mixture of

methyl salicylate and Paraplast, and the second a 25:75 mixture. This step was carried out on a gently rotating shaker-table to ensure an adequate replacement of the methyl salicylate by the Paraplast. Specimens were subsequently infiltrated in 100% Paraplast in a vaccum oven at 15 psi for no more than one hour. Infiltration times of more than one hour sometimes hardened the exoskeleton and made sectioning difficult. After infiltration, specimens were cooled to room temperature, and temporarily stored overnight.

A standardized orientation and embedding procedure was carried out on day four of the protocol schedule. Standardization allowed the genitalia to be cut in cross-section with reference to a known anatomical landmark, the relatively flattened dorsal surface of the ventral ovipositor valves. To accomplish this orientation, specimens previously infiltrated in Paraplast were placed under a dissecting microscope. Microsurgical scalpel and scissors were used to remove the dorsal ovipositor valves from the female without disturbing the coupled specimen. The firmness of the Paraplast supported the genitalia, and made this dissection possible. The dissection left intact the bursa, spermatheca, and aedeagus. Removal of the dorsal ovipositor exposed the relatively flat surface of the underlying ventral ovipositor, which was used as a reference plane during embedding and sectioning, as discussed below.

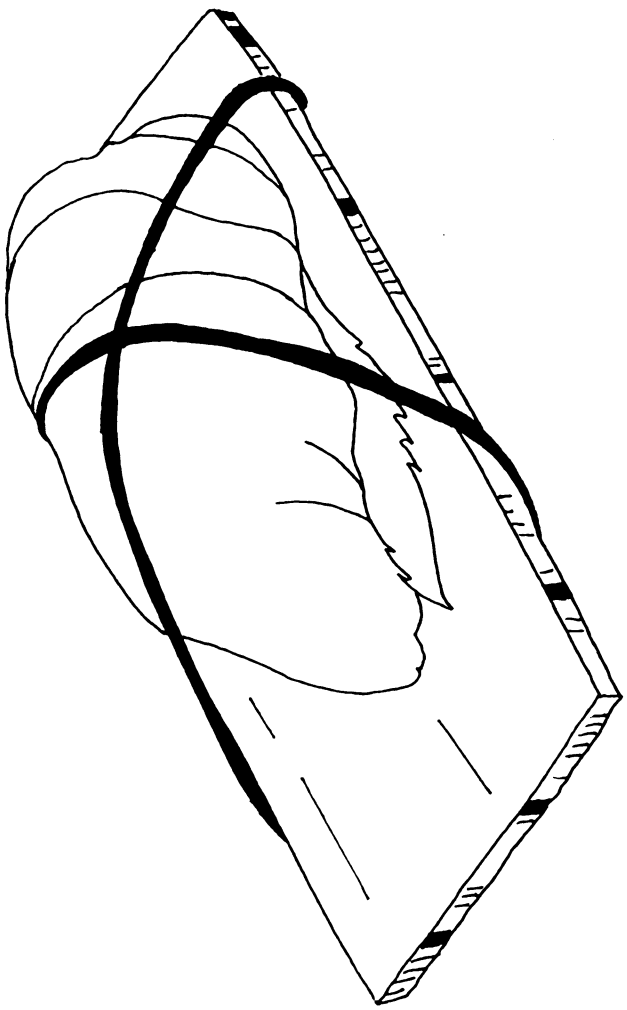
Prior to embedding, the exposed surface of the ventral ovipositors was painted black with acrylic paint to later identify the cleared specimen within the hardened Paraplast block. Specimens were inverted so the ventral ovipositors of the female rested flat on a narrow strip of plastic of known width, length, and height cut from a disposable embedding boat. In orienting the genitalia, the longitudinal,

transverse, and vertical axes of the specimen were aligned with the length, width, and height of the plastic strip, respectively (figure 6). Then the specimen was secured to the plastic strip with thread, so that the strip and the surface of the ventral ovipositors were parallel. Once securely fastened, the genitalia and plastic strip were placed in a disposable embedding boat with the bottom of the plastic strip flush against the bottom of the boat. The weight of the strip held the specimen down during the embedding process. Specimens in this standard orientation were infiltrated for 45 minutes in Paraplast, with the last 15 minutes under a vacuum of 15 psi.

After removal from the vacuum oven, the genitalia were left undisturbed in the embedding boat for approximately 90 seconds while the Paraplast soldified along the base of the specimen and plastic strip. This timed procedure secured the specimen to the strip and the strip to the bottom of the boat, but left the majority of the Paraplast in molten form. Before the Paraplast began to polymerize throughout, microsurgical scissors and forceps were warmed over an alcohol flame and used to remove the thread from the genitalia. (Instruments cooler than the Paraplast promoted polymerization). In this manner, the genitalia remained in standard orientation, firmly attached to the plastic strip along the bottom of the boat, while the anchoring thread was removed. Fully embedded specimens were removed from the boats and chilled in a refrigerator to further solidfy the Paraplast block. This made the densities of the Paraplast and exoskeletal material more nearly similar, and improved the cutting of the microtome blade.

When a specimen was ready for sectioning, the plastic strip abutting the embedded genitalia was peeled away from the block face,

Figure 6. Copulating genitalia attached to plastic strip before paraffin infiltration.



exposing the flat surface of the ventral ovipositors below. Removal of this strip also left a shallow impression of known length, width, and height in the face of the Paraplast block, just above the embedded genitalia. Blocks were mounted in the chuck of a rotary microtome with this impression and the underlying surface of the ventral ovipositors facing upward. Since the length, width, and height of the impression were parallel to the longitudinal, transverse, and vertical axes of the specimen, respectively, and since the plane of the impression was parallel to the surface of the ventral ovipositors, the orientation of the impression automatically oriented the specimen with respect to the ventral ovipositors. By manually adjusting the chuck holding the block in place, the impression and the genitalia were brought into alignment for cutting cross-sections. The blackened surface of the ventral ovipositor, clearly visible through the block, also aided in this orientation.

Cross-sections of the coupled genitalia were cut with a rotary microtome at 15 micron intervals and mounted on glass slides with egg albumin. During the winter months, static electricity in the laboratory caused the genitalic sections to curl and fall apart. A humidifier was added to the room to reduce this electrical charge and facilitate sectioning (Humason, 1979). Additionally during the winter, sections were suspended on a film of water over the slides, and then placed on a warming tray at 45 degrees centigrade for a minimum of twelve hours. The warm temperature and surface tension of the water helped restore the sections to their normal dimensions. The warm temperature also expanded and dispersed air bubbles under the sections which could cause specimens to fall out of the slide during staining procedures.

Specimens were stained with hematoxylin and Ponceau S according to the standard methods described in Humason (1979). Coverslips were placed over the slides with Permount to protect the sections. Serial cross-sections from each pair of copulating genitalia were stored for later digitization and analysis of genitalic fit.

Measuring genitalic incompatibility.

Operational definitions of genitalic incompatiblity should quantify the spatial relationships between the copulating genitalia that presumably affect the transfer of the spermatophore tube and the subsequent passage of sperm through the spermatophore tube into the spermathecal duct (Bennack, unpublished data; Ewen and Pickford, 1975; Gregory, 1965; Hartmann, 1970; Pickford and Gillot; 1971; Pickford and Padgham, 1973). In this study, geometric measures were considered the simplest and analytically most elegant means of quantifying the fit of the genitalia. According to Bookstein et al. (1985), geometric representations of biological forms more precisely capture the spatial relationships of complex morphologies than commonly used morphometric measures such as arbitrary distances (e.g. maximum lengths, widths, and heights), and composite statistical scores (e.g. principal components and factor axes).

Many geometric definitions of genitalic incompatibility could be formulated for Barytettix grasshoppers, but I chose to focus on three specific measures mentioned by Cohn and Cantrall (1974). These estimates are briefly described here, and discussed more fully below.

(1) Unoccupied bursal volume. A bursa incompletely filled by the aedeagus may increase the distance over which the spermatophore tube must maintain an optimal trajectory to reach the

spermathecal duct. Volumetric differences between the bursa and aedeagus best describe this type of incompatibility.

(2) Genitalic orientation. Misaligned genitalia may cause the spermatophore tube to travel toward the spermathecal duct at less than an optimal trajectory. In this case, incompatibility can be described by the relative alignment of the genitalia in a fixed coordinate system.

(3) Corresponding curvature. The lock-and-key hypothesis predicts complementarity in the shapes of the male and female genitalia. A close fit and precisely corresponding curvature should stabilize movements of the genitalia, thus facilitating successful transfer of the spermatophore tube. Incompatible curvature can be described by a landmark-free technique such as the medial axis function.

Cross-sections sampled and the scoring of spermatophore tube transfer.

All estimates of genitalic incompatibility were measured on cross-sections of the copulating genitalia taken at 15%, 30%, and 45% of the length of the bursa (measured from the exterior opening of the bursa to the beginning of the spermathecal duct). These distances provided a standardized sampling of genitalic fit at shallow, intermediate, and deep portions of the bursa, respectively. Aedeagal structures present at depths greater than 50% of the bursa were primarily apical, and generally too small to be analytically tractable. Since the apex of the ventral aedeagal valves often penetrated to depths greater than 80% of the bursa, aedeagal penetration was measured separately (discussed below).

A priori assumptions about what constitutes compatible versus incompatible fits served as analytical guidelines, but were not binding on the data analysis. Instead, multivariate analyses were used to judge the statistical significance of the measures of incompatibility in predicting the successful transfer of the spermatophore tube into the spermathecal duct (statistical procedures are discussed later).

The presence of sperm (insemination success) could not be reliably scored because sperm cells were generally destroyed by the KOH genitalic pretreatment. However the muco-protein spermatophore tube proved more durable. Since the transfer of the spermatophore tube into the spermathecal duct is probably critical to insemination in many grasshopppers (Ewen and Pickford, 1975; Gregory, 1965; Hartmann, 1970; Pickford and Gillot; 1971; Pickford and Padgham, 1973), the observation of spermatophore material in sections of the spermathecal duct and/or spermatheca was used as an indication of insemination success for a given mated pair. Spermatophore material was detected using the Ponceau S staining method of Humason (1979; see also Gregory, 1965). In prepared sections, the spermatophore tube stained yellow. This contrasted strongly with the spermatheca/spermathecal duct whose connective tissues stained red.

Volume of the female bursa unoccupied by the male aedeagus.

Perhaps the simplest estimate of genitalic incompatibility is the volume of space within the female bursa unoccupied by the male aedeagus. An excess amount of space between the bursal wall and the aedeagus may complicate the transfer of the spermatophore tube by increasing the distance over which the tube requires a constant trajectory (Cohn and Cantrall, 1974). If the male and female genital openings are too far

apart, the spermatophore tube may strike the bursal wall and fail to enter the spermathecal opening (observed by Pickford and Gillot, 1971, in Melanoplus). But an adequately occupied bursa may reduce destabilizing movements of the aedeagus, and thus ensure the passage of the spermatophore into the spermatheca. From this perspective, a precise volumetric fit may be operationally defined as a completely filled bursa such that no space within the bursa is left unoccupied by the male aedeagus.

In each sampled section, the volumetric fit of the aedeagus within the bursa was estimated as the difference between the cross-sectional areas of the two organs. To arrive at this measure, histological sections of the genitalia were projected at a magnification of 430 times, and traced on 12" x 18" sheets of blank newsprint. The cross-sectional areas of the genitalic tracings were then measured using a digital, polar-compensating planimeter at an accuracy of 0.1 square centimeters.

Relative orientation of the genitalia.

According to Cohn and Cantrall, the relative orientation of the male and female genitalia may determine whether the spermatophore tube is properly guided into the spermathecal duct. Adequately aligned genitalia should successfully transfer spermatophores, but misaligned male genitalia may pass spermatophores at less than optimal trajectories, thus reducing the likelihood of spermatophore transfer.

Quantifying the orientation of the male aedeagus relative to the surrounding female bursa involves several independent parameters, including translations along and rotations about the three-dimensional axes of each form. Estimating the relative alignment of male and female

structures along these axes would require more measurements than are feasible in a study of this scope. Instead, three orientation parameters of related biological meaning were estimated from the cross-sectional tracings of the genitalia (figure 7).

Upon each cross-sectional tracing, the major (latero-medial) and minor (dorso-ventral) axes of the bursa, dorsal aedeagal valves, and ventral aedeagal valves were drawn. The major axis intercepted the lateral-most points of each form, and the minor axis was the perpendicular bisector of the major axis. The major and minor axes of the female bursa provided a reference axial system for determining the relative orientation of the male valves. The major axis of the bursa was the absicssa (x-axis) of the reference system, the minor axis was the ordinate (y-axis), and the intersection of the major and minor bursal axes provided the origin of the female reference system.

Polar coordinates were used to describe the location of the aedeagal valves with respect to the bursal origin. Since the male polar coordinates were always with reference to the positive x-axis of the bursa, the coordinate location of the valves is sometimes referred to as the standard location of the aedeagal valves. Orientation of the dorsal and ventral aedeagal valves with respect to the female axial system was measured as follows:

(1) Polar coordinate distance of the valves. The distance from the origin of the major and minor axes of the bursa, to the origin of the aedeagal valves. (Dorsal and ventral valves were measured separately.)

(2) Polar coordinate angle of the valves. The angle formed between the positive x-axis of the bursal system, and the ray extending

from the bursal origin through the origin of the aedeagal valves. (Dorsal and ventral valves were measured separately.)

The third measurement represents the rotation of the male axial system with respect to the female axial system.

(3). Rotation of the valves. The inclination or declination of the major axis of the aedeagal valves relative to the positive major axis of the bursa. (Dorsal and ventral valves were measured separately.)

All estimates of orientation were measured using a compass and ruler. Although a time-consuming process, hand measurement provided a visual familiarity with the fit of the genitalia that complemented the digital representations of genitalic fit provided by the medial axis function (discussed below).

Corresponding curvature of the genitalia.

In areas where the aedeagal valves were in close proximity to the bursal wall, the medial axis function (Bookstein, et al., 1985) was used to characterize the relative curvature of the exterior surface of the valves with respect to the interior bursal wall (figures 7 and 8). According to Bookstein et al., the medial axis is one of the few geometric techniques available for analyzing morphologies without obvious landmarks. A lack of landmarks was notable for the smoothly curving surfaces of the bursa and aedeagal valves when viewed in cross-section.

The medial axis function measures the space between the aedeagal valves and the bursa as the set of the foci (centers) of circles whose radii touch male and female structures only once each (figure 8). The

Figure 7. Serial cross-sections of male and female genitalia of Barytettix humphreysii humphreysii while copulating. From front to back these sections were taken at 15%, 30%, and 45% of the depth of the bursa.

The depth of penetrance of the aedegal valves was measured from the posterior of the bursa, to the point where the aedeagal apex disappeared from cross-sections. The unoccupied bursal volume at each depth was estimated from its cross-sectional area. To measure the relative orientation of the genitalia, dorso-ventral and latero-medial axes of symmetry were constructed for the bursa, the dorsal valves, and the ventral valves; then the polar coordinates of the male valves were measure with respect to the origin of the bursal reference system. The rotation of the male valves was measured as the angle of intersection between the male and female latero-medial axes.

Note that in this specimen, the center of the ventral valves was much closer than the dorsal valves to the origin of the bursal reference system. The ventral valves were rotated with respect to the bursa, but this rotation became less pronounced deeper into the bursa. The dorsal valves were relatively unrotated at all bursal depths.
Symbols used in the figure:

BR -bursa

DV -dorsal valve

VV -ventral valves

1 -axes of symmetry, ventral valves

2 -axes of symmetry, bursa

3 -axes of symmetry, dorsal valves

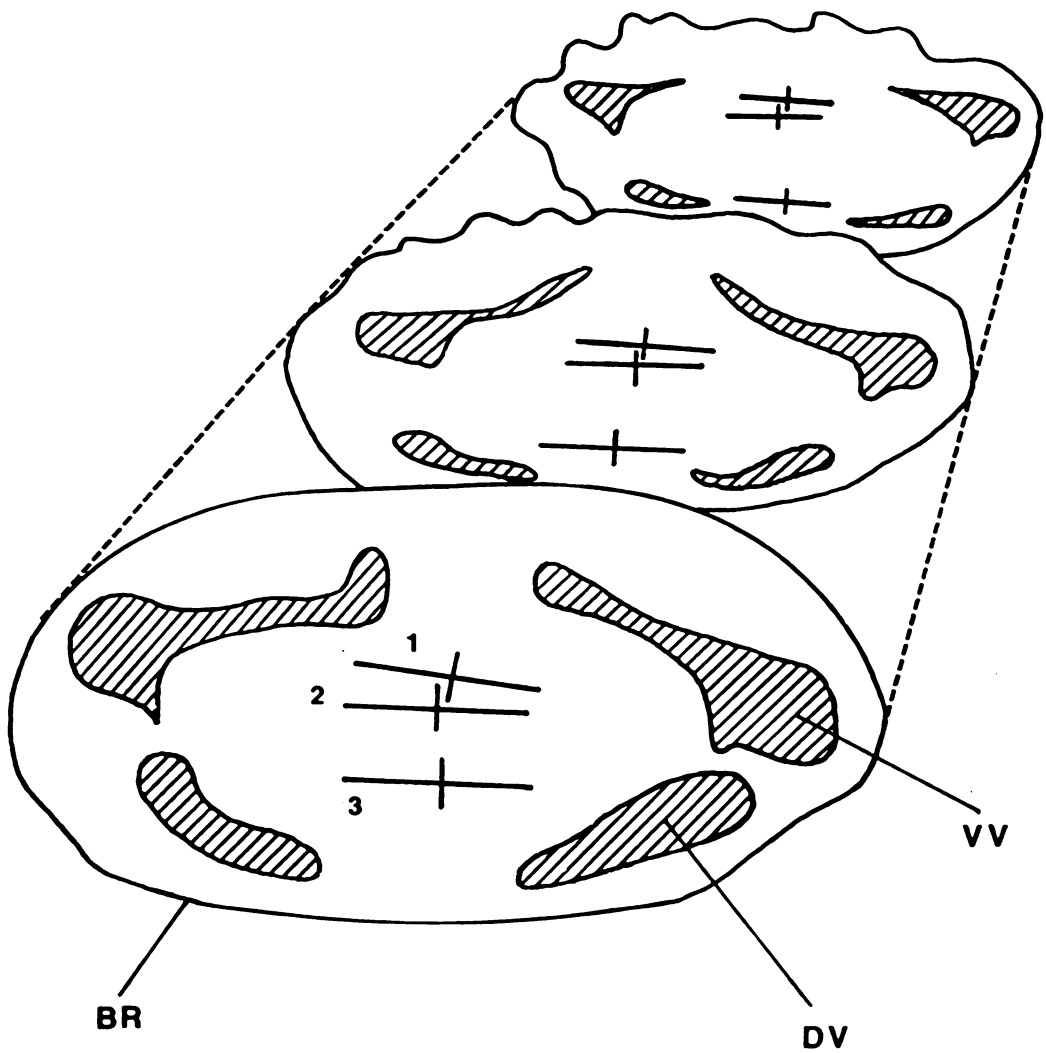

1
2
3
VV
BR
DV

Figure 8. The medial axis function (Bookstein, et al., 1985) used to measure the correspondence in the curvature between the bursal wall and aedeagal valves. The function is illustrated only for the bursa and right dorsal aedeagal valve. The medial axis function described the distance between the aedeagal valve and the bursa as the set of the centers of all circles touching each male and female structure only once. A precise fit would be indicated by small and invariant radial distances along the entire length of the medial axis.

Symbols used in the figure:

BR -bursa

DV -dorsal valve

MA -medial axis

RD -radial distance

foci of the circles define a medial axis of symmetry in the space between the male and female forms. The radii describe the distance from the medial axis out to corresponding male and female boundary points. [By definition, each radius must intersect the boundary curve to form a right angle with a line drawn tangent to the intercepted local curvature (Bookstein et al., 1985).] The radii of these circles will be unequal as long as the corresponding curves of the aedeagus and bursa change at different rates. A lack of fit between male and female genital curvature would be indicated by variable radial distances along the medial axis. In contrast, a precisely curving fit of the genitalia would be described by an invariant radial distance along the entire length of the medial axis.

Cross-sectional outlines of the genitalia in copula were digitized to provide the raw data for the medial axis analysis. Video images of the histological cross-sections were digitized using a program developed by Ben Vernia, and made available through William Fink. This Turbo Pascal program currently runs on IBM and IBM-compatible microcomputers. The medial axis function was calculated according to the algorithm of Bookstein et al. (1985). The medial axis program used in this study was written in Turbo Pascal for the Victor 9000 micro-computer by Robert Kriegel and Donald Straney. Estimates of genitalic incompatibility derived from the medial axis function include:

(1) Average radial distance. The average radial distance from the medial axis to corresponding points on the bursa and aedeagal valves, summed over all medial axis foci. According to the lock-and-key hypothesis, the aedeagus should fit closely against the bursa and the average radial distance should be small.

(2) Variation in the radial distance. The standard deviation in the radial distance between the bursa and aedeagal valves. The standard deviation measures variation in radial distances resulting from unequal curvature along corresponding portions of the aedeagus and bursa. According to the lock-and-key hypothesis, these radial distances should be invariant if the aedeagus and bursa are curving in parallel.

(3) The medial axis length. The arc length of the medial axis measures the distance over which the bursa and aedeagal valves are in physical proximity. In regression analyses, the slope and significance of this variable indicate whether curving fit is more important in areas of broad or limited genitalic contact. (The Kriegel/Straney algorithm terminates the medial axis with endpoints, provided the space between the bursa and aedeagus is closed laterally and medially with single digitized points representing the vertices of isosceles triangles. Axis endpoints represent regions where the lateral and medial edges of the aedeagus no longer face the bursal wall, but are turned toward the interior of the bursa.)

Body size differences and the depth of aedeagal penetration.

In addition to the volumetric fit, relative orientation, and corresponding curvature of the aedeagus and bursa, the following overall estimates of mating incompatibility were scored for each copulating pair:

(4) Body size differences. Sexual dimorphism in body size (males were smaller than females) was a fundamental contributor to spermatophore transfer success in intra-racial crosses of B.

humphreysii. Very small males had noticeable difficulty extending the aedeagus around the female abdomen to penetrate the bursa (Appendix I). Additionally, differences in overall body size were evident between the races in both sexes. B. h. humphreysii is a larger race than B. h. cochisei (Cohn and Cantrall, 1974). Inter-racial size differences dramatically affected the ability of males to transfer spermatophore tubes to females in the reciprocal crosses of this experiment. Differences in male and female body size were expressed as the differences in their respective masses.

(5) Aedeagal penetration/distance between the genital openings. The distance between the aedeagal opening (phallotreme) and the orifice of the spermathecal duct was used as an indication of the minimum distance the spermatophore must travel to reach the spermathecal opening. The distance between these openings was calculated by adding the number of slides (at 15 micron intervals) between the phallotreme and the spermathecal opening. The opening of the phallotreme was operationally defined as the point where the apex of the aedeagus disappears from cross-sections, but the location of the spermathecal opening was more difficult to establish. The spermathecal opening was arbitrarily defined as the beginning of a noticeable and consistent reduction in the diameter of the bursa as it graded into the spermathecal duct over a distance equal to or greater than 45 microns. The distance between the phallotreme and the spermathecal duct opening was directly related to the depth of aedeagal penetration and these two terms are often used interchangeably in the text. The depth of

aedeagal penetration was simply the length of the bursa minus the distance between the phallotreme and the spermathecal duct opening.

Statistical methods.

Multiple regression, multivariate analysis of covariance, discriminant analysis, and principal component clustering were used to assess genitalic fit and its relationship with spermatophore transfer success. All genitalic variables were log-transformed to adjust for scaling effects and to linearize the covariance structure of the data (Bookstein et al., 1985; Sokal and Rohlf, 1981). Because some estimates of genitalic fit were measured in incomparable units (e.g. degrees vs. distance measures), multivariate statistical analyses were performed on the correlation matrix (Bookstein et al., 1985; Strauss, personal communication).

Each specimen had as many as three data entries associated with a given genitalic variable. These data points corresponded to estimates of genitalic fit measured at 15%, 30%, and 45% of the bursa, respectively. Each estimate of genitalic fit was an average value based on measurements across the different standard depths of the bursa. This analytical approach, the equivalent of assessing the global fit of the genitalia, revealed significant differences among the crosses. Consequently, a section-by-section analysis of genitalic fit was not performed.

Multivariate statistical procedures were implemented on SAS Version 5.16 (SAS Institute, 1985) installed on the VAX 11/730 computer in the Department of Entomology, Michigan State University. The SAS data handling routine automatically dropped any histological sections having one or more missing data values.

Cluster analysis

Cluster analysis was used in conjunction with a visual inspection of the slides to determine how well the genitalia fit together among the different crosses. Crosses with high spermatophore transfer frequencies were expected to show consistency in the location, orientation, and curvature of the aedeagal valves within the bursa, while crosses with poor spermatophore transfer success should be more variable in this regard. In addition, successful crosses should share many common clusters of genitalic variables, provided that patterns of statistical covariation are associated with a functionally efficient copulation (after Olson and Miller, 1958). Finally, clusters common to the successful crosses should be broken apart (covariation disrupted) in the functionally inefficient, unsuccessful crosses.

The VARCLUS procedure (SAS Institute, 1985) was used to divide the estimators of genitalic fit within each mating cross into non-overlapping clusters. Within each cluster, the VARCLUS procedure computed a linear combination of variables equivalent to the first within-cluster principal component. PROC VARCLUS then attempted to maximize the sum, across the clusters, of the variance associated with the original variables.

VARCLUS is a type of oblique component analysis closely related to multi-group factor analysis (Harman, 1976). As a variable reduction technique, the clusters produced by PROC VARCLUS generally do not explain as much variance as an equal number of principal components. But the clusters produced by VARCLUS are generally easier to interpret than principal components, or even rotated principal components (SAS Institute, 1985). The statistical co-variation explained by clustered genitalic variables was used to supplement visual inspection of how well

the genitalia fit together.

Cluster groupings were compared among crosses to investigate the level of cluster concordance. According to the lock-and-key hypothesis, crosses with similar spermatophore transfer frequencies should have genitalia that fit together in comparable ways. Crosses with equivalent spermatophore transfer frequencies should also covary in their genitalic measures in ways that can be explained by similar cluster groupings. In addition, dissimilar clusters among crosses may suggest morphological regions or functional components of the genitalia where genitalic fit is fundamentally different.

By default, VARCLUS starts with all variables in one cluster, and then repeats the following steps to form new clusters:

(1) A cluster is chosen for splitting if it has either the smallest percentage of variation explained by any cluster or the largest second eigenvalue.

(2) The chosen cluster is split into two new clusters using an orthoblique rotation to find the first two principal components of the original cluster. Then each variable is assigned to the rotated first or second principal component with which it has the highest squared correlation. Thus, the members of the two new clusters are variables associated with either the first or second principal component of the original cluster.

(3) Variables are iteratively reassigned to clusters until the variance accounted for by the cluster components is maximized.

Only the default initialization (nearest component sorting method) was used to perform variable reassignment during step three. With the default option, once a chosen cluster is split, variables can not be re-tested to see if assigning them to a different cluster increases the

amount of variation explained. In most cases, however, the variance maximization of the nearest component sorting method cannot be improved by re-testing variables in alternative clusters (SAS Institute, 1985).

Discriminant analysis

The STEPDISC procedure (Sas Institute, 1985) was used to select a subset of measures of genitalic fit that discriminated between successful and unsuccessful copulating pairs within each mating cross. Significant discriminators of spermatophore transfer success identify aspects of genitalic fit that are probably associated with morphological incompatibililty.

Discriminant analysis within each mating cross was done according to the backward elimination option of PROC STEPDISC. Backward elimination began with all variables in the model. At each subsequent step, the one variable contributing least to the discriminatory power of the model (as measured by Wilks' lambda) was selectively removed. The original variables in the model were: body size differences between copulating males and females, unoccupied bursal area, dorsal and ventral valve penetrations, dorsal and ventral valve polar coordinate distances, dorsal and ventral valve polar coordinate angles, dorsal and ventral valve rotations, average radial distances associated with the dorsal and ventral valves, variation in the radial distances associated with dorsal and ventral valves, and medial axis lengths associated with the dorsal and ventral valves.

Variables were chosen to leave the model based on an analysis of covariance at the 0.01 level of significance. In this procedure, variables already in the discriminant model acted as covariates, and the variable under consideration for removal served as the dependent

variable. When all remaining variables met the criterion to stay in the model, the backward elimination process stopped.

Discriminant analysis was particularly useful in assessing the influence of male and female size differences upon the other measures of genitalic fit. By stipulating that the discriminant model always include male and female size differences, only those variables that further maximized the variance between successful and unsuccessful groups were allowed to remain. In this manner, genitalic variables highly correlated with body size differences were eliminated from the model, but variables relatively independent of size effects remained.

Multivariate analysis of covariance

The multivariate analysis of covariance (MANCOVA) tests a suite of dependent variables for homogeneity among treatment means in a desgin similar to the closely related multivariate analysis of variance (MANOVA). However, before treatment means are tested in a MANCOVA, they are adjusted for differences among the treatments caused by one or more independent variables known as covariates.

In MANOVA, the extent to which variation in the dependent variables distinguishes the treatment groups is determined by a statistical test of significance: a multivariate equivalent of the univariate F-ratio test. In a fashion analogous to the univariate F-test, the sum-of-squares-and-cross-products (SSCP) matrix for the treatment effects is "divided" by the SSCP matrix for the error effects. (The inverted SSCP error matrix is post-multiplied by the SSCP treatment matrix.) A new matrix results that enables an investigator to utilize probability expectations to assess the reliability of the observed differences between the treatment groups.

MANCOVA performs the same test of significance between treatment means as MANOVA. But before treatment differences are tested, multiple regression techniques are used to statistically control the covariate effects. A covariate is any continuous variable that is associated with one or more of the dependent variables. If a great deal of variation in the dependent variables is associated with a covariate, then this "noisy background" produces a high SSCP error matrix against which only extremely large differences in the treatment groups can be detected. Adjustment for the effects of covariates allows for a more precise test of the differences between treatment groups.

In this study, differences in male and female body size were thought to influence many of the estimates of genitalic fit. MANCOVA was used to assess the influence of size differences upon these genitalic variables in the following way. If the significance of any genitalic measure was removed by adjusting for male and female size differences, and provided the MANCOVA test showed significant differences between the successful and unsuccessful groups, then male and female body size differences were assumed to underlie the effect of that genitalic measure in determining spermatophore transfer success.

The significance of individual genitalic variables in the MANOVA was judged at the 0.03 level of significance. In the MANOVA, statistical significance of the genitalic variables, independent of body size differences, was judged at the 0.01 level. The univariate method of evaluating the individual dependent variables has been justified by Hummel and Sligo (1971). These investigators used computer simulations to demonstrate that, given a significant overall MANOVA, an analysis of variance for each dependent variable resulted in significance values close to the highly conservative classical methods of variable testing.

Barker and Barker (1984) also argue, assuming a significant MANOVA test, that an analyst is free to apply the univariate analysis of variance to the separate dependent variables with the assurance that the comparisons are controlled at approximately the chosen alpha level.

MANCOVA tests were performed for each mating cross using the GLM procedure (SAS Institute, 1985), and specifying the MANOVA and univariate statistic options.

Multiple regression

In this study, discriminant analysis and MANCOVA tests revealed that differences in male and female body size, along with certain orientation parameters, were primarily associated with spermatophore transfer success. To better understand how size might contribute to a lock-and-key fit, an exploratory multiple regression technique was used to investigate the relationship between male and female size differences and spermatophore transfer success.

Copulating pairs within each cross were ranked (PROC RANK; SAS Institute, 1985) according to male and female body size difference, and then grouped into three size classes of approximately equal number (the smallest class had ten members.) PROC MEANS was used to calculate the average spermatophore transfer frequency for each size class based on individual scores of spermatophore transfer. The appropriate spermatophore transfer frequency was reassigned to each copulating pair according to its class membership. In this manner, continuous measures of male and female size differences were associated with conceptually-continuous spermatophore transfer frequencies deliberately biased to reveal body size effects. This biasing technique increased the resolving power of the multiple regression with respect to body size

differences, but at the expense of the other genitalic variables in the analysis. Since the effects of other genitalic variables were accounted for in the discriminant and MANCOVA analyses, this size-biased regression was justified to the extent that only body size effects were compared among the crosses.

PROC GLM (SAS Institute, 1985) was used to perform multiple regressions upon the size-biased data set. The importance of each variable was tested at the 0.01 level of significance, holding the other variables constant. Using standard regression coefficients permitted within-cross comparisons of the importance of each genitalic variable, but these comparisons must be cautiously interpreted with the biased data. Instead, the main utility of this approach was in comparing the regression slopes of body size differences among the intra-racial and inter-racial crosses.

CHAPTER 4

RESULTS AND DISCUSSION OF THE MATING EXPERIMENTS

Genitalic fit and spermatophore tube transfer: an overview of results.

The classical interpretation of the lock-and-key hypothesis predicts that genitalic incompatibility can lead to mechanical reproductive isolation between closely related taxa. Inter-taxon transfer of the spermatophore tube should be impossible when male and female genital morphologies are completely mismatched. But when taxa are incompletely isolated (as may be characteristic of B. humphreysii races), genitalic incompatiblity should reduce but not entirely eliminate the possibility of inter-taxon spermatophore transfer. Transfer frequencies should correspond to the degree of compatiblity between male and female genitalia, regardless of the identity of the mated pair.

According to the lock-and-key hypothesis and based on the work of Cohn and Cantrall, the following predictions can be made about spermatophore transfer frequencies. Intra-racial crosses of B. h. humphreysii and B. h. cochisei should be mechanically and morphologically compatible, resulting in essentially equivalvent transfer frequencies. But in inter-racial crosses, the mismatched genitalia should fit together poorly, resulting in transfer frequencies lower than those in the intra-racial crosses.

The analysis of spermatophore transfer frequencies indicated the intra-racial crosses of B. humphreysii were equally capable of spermatophore transfer. B. h. humphreysii females received spermatophore tubes 83.87 percent of the time from B. h. humphreysii males (the HMHF cross: successful, n = 26; unsuccessful, n = 5), and B.

h. cochisei females received spermatophores 83.87 percent of the time from B. h. cochisei males (the CMCF cross: successful, n = 26; unsuccessful, n = 5). These identical frequencies agree with the prediction of the lock-and-key hypothesis that the intra-racial crosses should be morphologically compatible, and therefore equally successful.

While the intra-racial transfer frequencies agreed with the lock-and-key hypothesis, inter-racial crosses showed an unexpected asymmetry in spermatophore transfer frequencies. B. h. cochisei females received spermatophores 90.32 percent of the time from B. h. humphreysii males (the HMCF cross: successful, n = 28; unsuccessful, n = 3), but B. h. humphreysii females received spermatophores only 57.89 percent of the time from B. h. cochisei males (the CMHF cross: successful, n = 22; unsuccessful, n = 16). The low frequency of spermatophore transfer in the CMHF cross was consistent with the lock-and-key hypothesis, but the unexpectedly high transfer frequency in the HMCF cross was counter to hypothetical expectations. Despite the mismatch of male and female genitalia predicted by Cohn and Cantrall, the HMCF cross was more successful than the intra-racial crosses in the transfer of spermatophore material.

These findings raise a number of important questions about the morphology of genitalic fit in Barytettix grasshoppers, for example:

(1) Do the genitalia in the HMCF cross function in a way similar to the genitalia in the intra-racial crosses?

(2) Why do genitalia appearing morphologically incompatible in both inter-racial crosses only mis-function in the CMHF cross?

(3) Does the lower transfer frequency in the CMHF cross result from a fundamentally different genitalic fit than the other crosses?

(4) Do asymmetric transfer frequencies among inter-racial crosses

refute the lock-and-key hypothesis, or is mating compatibility structured at another level of biological causation that can adequately explain all four crosses?

In addressing each of these questions I will develop the main thesis of this work: that associations between genitalic fit and spermatophore transfer success are often structured by body size differences between the copulating males and females.

The overall effect of male and female body size differences.

Sexual dimorphism in body size is prevalent in both races of B. humphreysii, with females generally larger than males. In addition, B. h. humphreysii is a larger race than B. h. cochisei in both sexes. [For data related to these points, refer to size measurements reported by Cohn and Cantrall (1974).]

Sexual dimorphism was also evident in the present study (Table 1). Among all the intra-racial pairs reported, females were always larger than their respective male partners. In the HMHF cross, males and females weighed an average of 638.71 mg (SD = 92.46 mg) and 1216.45 mg (SD = 197.16 mg), respectively. In the CMCF cross, males and females averaged 486.77 mg (SD = 68.97 mg) and 973.23 mg (SD = 147.13 mg), respectively. These measurements also suggest, in both sexes, that B. h. humphreysii is a larger race than B. h. cochisei.

Females were larger than males in the inter-racial pairs (Table 1), but sexual size differences were greater in the CMHF cross and smaller in the HMCF cross, than the size differences observed in the intra-racial crosses. In the CMHF cross, B. h. cochisei males and B. h. humphreysii females averaged 467.11 mg (SD = 81.60 mg) and 1138.95 mg (SD = 193.74 mg), respectively. Clearly humphreysii females were much

Table 1. Summary statistics for genitalic and body size variables among crosses of B. humphreysii. (Means, standard deviations, coefficients of variation, and number of histological sections were scored for each variable and pooled over successful and unsuccessful transfer of the spermatophore tube. Sections were cut at 15%, 30%, and 45% of the bursa, so that the maximum number of sections possible per cross was three times the number of copulating pairs. See the accompanying legend.)

	HMHF	CMCF	HMCF	CMHF
num. copulating pairs				
	n = 31	n = 31	n = 31	n = 38
max. number sections				
	n = 93	n = 93	n = 93	n = 114
male mass				
	638.71	486.77	616.45	467.11
	(92.46)	(68.97)	(67.98)	(81.60)
	14.48%	14.17%	11.03%	17.47%
	n = 93	n = 93	n = 93	n = 114
female mass				
	1216.45	973.23	1013.55	1138.95
	(197.16)	(147.13)	(180.77)	(193.74)
	16.21%	15.12%	17.84%	17.01%
	n = 93	n = 93	n = 93	n = 114
size diff				
	577.75	483.53	397.09	671.84
	(190.47)	(145.95)	(187.77)	(210.44)
	32.97%	30.18%	47.29%	31.32%
	n = 93	n = 93	n = 93	n = 114
bursa length				
	355.16	498.39	496.94	348.95
	(81.30)	(124.64)	(70.81)	(84.43)
	22.89%	25.01%	14.25%	24.20%
	n = 93	n = 93	n = 93	n = 114
dv to sp				
	96.77	222.58	124.36	233.29
	(51.62)	(90.12)	(67.47)	(86.08)
	53.34%	40.49%	54.25%	36.90%
	n = 93	n = 93	n = 93	n = 114

Table 1. continued.

	HMHF	CMCF	HMCF	CMHF
vv to sp				
	51.29	66.29	94.42	105.40
	(49.42)	(53.73)	(58.84)	(89.30)
	96.35%	81.05%	62.32%	84.72%
	n = 93	n = 93	n = 93	n = 114
unc bur				
	97.76	88.23	103.29	80.41
	(18.73)	(13.10)	(16.36)	(24.57)
	19.16%	14.85%	15.84%	30.56%
	n = 93	n = 93	n = 93	n = 114
dv bur avg				
	14.20	15.54	14.72	9.65
	(5.23)	(5.58)	(4.13)	(4.95)
	36.83%	35.91%	28.06%	51.30%
	n = 85	n = 80	n = 87	n = 44
dv bur var				
	3.60	3.63	4.39	2.30
	(2.16)	(2.09)	(2.21)	(1.74)
	60.00%	57.58%	50.34%	75.65%
	n = 85	n = 80	n = 87	n = 44
dv axs				
	242.44	203.77	290.62	175.63
	(58.08)	(83.34)	(54.57)	(91.75)
	23.96%	40.90%	18.78%	52.24%
	n = 85	n = 80	n = 87	n = 44
vv bur avg				
	20.09	27.30	23.41	24.53
	(8.08)	(8.75)	(7.78)	(10.57)
	40.22%	32.05%	33.23%	43.09%
	n = 89	n = 91	n = 90	n = 85
vv bur var				
	7.52	10.00	8.41	9.56
	(3.98)	(4.29)	(3.45)	(4.65)
	52.93%	42.90%	41.02%	48.64%
	n = 89	n = 91	n = 90	n = 85

Table 1. continued.

	HMHF	CMCF	HMCF	CMHF
vv axs				
	311.63	331.58	366.62	271.62
	(89.83)	(105.19)	(87.36)	(106.02)
	28.83%	31.72%	23.83%	39.03%
	n = 89	n = 91	n = 90	n = 85
dv pol dis*				
	10.97	12.12	11.52	11.10
	(2.29)	(3.30)	(2.12)	(2.50)
	-----	-----	-----	-----
	n = 85	n = 79	n = 86	n = 45
dv pol ang*				
	268.55	271.61	269.15	269.66
	(9.05)	(7.93)	(7.80)	(13.03)
	-----	-----	-----	-----
	n = 85	n = 79	n = 86	n = 45
vv pol dis*				
	1.82	3.60	2.71	3.47
	(1.86)	(2.80)	(1.91)	(2.88)
	-----	-----	-----	-----
	n = 89	n = 91	n = 90	n = 92
vv pol ang*				
	181.05	233.80	222.73	189.22
	(102.87)	(83.18)	(83.67)	(91.62)
	-----	-----	-----	-----
	n = 77	n = 88	n = 87	n = 83
dv rot*				
	0.82	0.39	0.18	0.44
	(1.94)	(2.98)	(2.00)	(3.58)
	-----	-----	-----	-----
	n = 85	n = 79	n = 86	n = 45
vv rot*				
	0.26	0.88	0.31	0.42
	(2.01)	(3.22)	(1.85)	(4.74)
	-----	-----	-----	-----
	n = 89	n = 91	n = 90	n = 92

Table 1. continued.

Legend:

size diff = female mass minus male mass in milligrams.
dv to sp = distance between apex of dorsal valves and opening of spermathecal duct in microns.
vv to sp = as in dv to sp, but for ventral valves.
unc bur = unoccupied bursal area in microns squared.
dv axs = dorsal valve medial axis length in microns.
vv axs = as in dv axs, but for ventral valves.
dv bur avg = average radial distance in microns from the medial axis to the bursa and dorsal valves.
dv bur var = variation (standard deviation) in the radial distance from the medial axis to the bursa or dorsal valves.
vv bur avg = as in dv bur avg, but for the ventral valves.
vv bur var = as in dv bur var, but for the ventral valves.
dv pol dis = the distance in microns between the origin of the bursal polar coordinate system and the origin of the dorsal valve reference system.
dv pol ang = the angle in degrees formed between the positive x-axis of the bursal polar coordinate system, and the ray projecting from the origin of the bursal system through the origin of the dorsal valve reference system.
vv pol dis = as in dv pol dis, but for the ventral valves.
vv pol ang = as in dv pol var, but for the ventral valves.
dv rot = the angle of intersection in degrees between between the positive x-axis of the bursal polar coordinate system and the major axis of the dorsal valve reference system.
vv rot = as in dv rot, but for the ventral valve reference system.

*

Since the orientation measurements represented direction and not size, coefficients of variation were inappropriate for comparing variability. Instead, standard deviations may be compared directly among and within the crosses.

larger than cochisei males when compared to the intra-racial crosses. In the HMCF cross, B. h. humphreysii males were much closer to the size of B. h. cochisei females than is typical in the intra-racial crosses. In the HMCF cross, males and females weighed 616.45 mg (SD = 67.98 mg) and 1013.55 mg (SD = 180.77 mg), respectively.

During copulation, differences in body size clearly affected the ability of smaller males to reach around the abdomen and penetrate the bursa of larger females (Appendix I). In general, mounting attempts took longer, and females more frequently dislodged smaller males, than males closer to their own size. Male and female size differences also visibly affected genitalic fit. For example, the distance between the aedeagal and spermathecal duct openings (i.e. the depth of penetration) was greater in the CMHF cross when compared to the other three crosses.

Much of this chapter attempts to document how body size differences influenced genitalic incompatibility to produce asymmetric frequencies of spermatophore transfer in the inter-racial crosses. For example, in the HMCF cross the aedeagus of the relatively large male often fit snuggly and deeply within the bursa of the female. Large male size and a deep penetration may have overcome genitalic incompatibility by placing the aedeagal and spermathecal openings close together, increasing the likelihood of spermatophore transfer (compare values among crosses in Tables 1 and 2). In the CMHF cross, the aedeagus of the relatively small male only shallowly penetrated the bursa of the female. Small male size and shallow penetration in the CMHF cross probably compounded the mismatch of the genitalia, placing the male and female openings farther apart, and decreasing the likelihood of spermatophore transfer (compare Tables 1 and 2).

Table 2. Summary statistics for genitalic and body size variables within crosses of B. humphreysii. (Means, standard deviations, coefficients of variation, and number of histological sections were scored for each variable and grouped according to successful or unsuccessful transfer of the spermatophore tube. Sections were cut at 15%, 30%, and 45% of the bursa, so that the maximum number of sections possible per cross was three times the number of copulating pairs.

	HMHF	CMCF	HMCF	CMHF
num. copulating pairs				
succ.	n = 26	n = 26	n = 28	n = 22
unsucc.	n = 5	n = 5	n = 3	n = 16
max. number sections				
succ.	n = 78	n = 78	n = 84	n = 66
unsucc.	n = 15	n = 15	n = 9	n = 48
male mass				
succ.	632.69	486.67	615.71	458.18
	(87.54)	(68.41)	(71.00)	(84.45)
	13.84%	14.06%	11.53%	18.43%
	n = 78	n = 78	n = 84	n = 66
unsucc.	670.00	487.50	623.33	479.38
	(113.01)	(75.81)	(27.84)	(76.67)
	16.87%	15.55%	4.47%	15.99%
	n = 15	n = 15	n = 9	n = 48
female mass				
succ.	1227.31	984.81	1002.86	1133.18
	(208.23)	(154.18)	(180.76)	(217.28)
	16.97%	15.66%	18.02%	19.17%
	n = 78	n = 78	n = 84	n = 66
unsucc.	1160.00	895.00	1133.33	1146.88
	(113.58)	(22.76)	(156.20)	(157.60)
	9.79%	2.54%	14.03%	13.74%
	n = 15	n = 15	n = 9	n = 48

Table 2. continued.

	HMHF	CMCF	HMCF	CMHF
size diff				
succ.	594.62	498.15	387.14	675.00
	(195.98)	(163.26)	(191.16)	(233.03)
	32.96%	32.77%	49.38%	34.52%
	n = 78	n = 78	n = 84	n = 66
unsucc.	490.00	407.50	490.00	667.50
	(161.82)	(55.94)	(156.12)	(179.38)
	33.02%	13.73%	31.86%	26.87%
	n = 15	n = 15	n = 9	n = 48
bursa length				
succ.	356.54	502.22	498.75	344.32
	(82.51)	(127.10)	(72.21)	(90.74)
	23.14%	25.31%	14.48%	26.36%
	n = 78	n = 78	n = 84	n = 66
unsucc.	348.00	472.50	480.00	355.31
	(76.95)	(107.69)	(56.62)	(75.36)
	22.11%	22.79%	11.80%	21.21%
	n = 15	n = 15	n = 9	n = 48
dv to sp				
succ.	98.65	230.56	116.79	223.64
	(50.97)	(89.83)	(64.19)	(103.91)
	51.66%	38.96%	54.97%	46.47%
	n = 78	n = 78	n = 84	n = 66
unsucc.	87.00	168.75	195.00	246.56
	(55.03)	(91.60)	(98.08)	(61.57)
	63.25%	54.28%	50.30%	24.97%
	n = 15	n = 15	n = 9	n = 48
vv to sp				
succ.	55.38	72.22	87.32	107.73
	(53.58)	(58.68)	(56.76)	(90.53)
	96.75%	81.25%	65.00%	84.04%
	n = 78	n = 78	n = 84	n = 66
unsucc.	30.00	26.25	140.00	102.19
	(27.77)	(27.97)	(78.30)	(87.61)
	92.58%	106.56%	55.93%	85.73%
	n = 15	n = 15	n = 9	n = 48

Table 2. continued.

	HMHF	CMCF	HMCF	CMHF
unc bur				
succ.	98.77	88.74	104.29	85.22
	(17.87)	(13.39)	(15.67)	(27.13)
	18.09%	15.08%	15.03%	31.83%
	n = 78	n = 78	n = 84	n = 66
unsucc.	92.50	84.79	93.96	73.80
	(22.66)	(10.78)	(20.47)	(18.89)
	24.50%	12.72%	21.79%	25.59%
	n = 15	n = 15	n = 9	n = 48
dv bur avg				
succ.	14.35	14.93	14.72	9.06
	(5.37)	(5.68)	(3.88)	(3.82)
	37.43%	38.07%	26.37%	42.14%
	n = 70	n = 65	n = 82	n = 27
unsucc.	13.50	19.02	14.68	10.59
	(4.60)	(3.37)	(7.83)	(6.37)
	34.01%	17.74%	53.33%	60.17%
	n = 15	n = 15	n = 5	n = 17
dv bur var				
succ.	3.73	3.39	4.40	2.11
	(2.22)	(1.88)	(2.22)	(1.52)
	59.58%	55.37%	50.47%	72.01%
	n = 70	n = 65	n = 82	n = 27
unsucc.	3.03	4.98	4.09	2.59
	(1.77)	(2.76)	(2.21)	(2.05)
	58.61%	55.34%	54.06%	79.32%
	n = 15	n = 15	n = 5	n = 17
dv axs				
succ.	246.58	195.10	292.17	189.16
	(60.28)	(82.27)	(53.08)	(101.41)
	24.45%	42.16%	18.17%	53.61%
	n = 70	n = 65	n = 82	n = 27
unsucc.	223.13	252.86	265.18	154.14
	19.26%	29.51%	29.52%	46.38%
	(42.96)	(74.61)	(78.27)	(71.49)
	n = 15	n = 15	n = 5	n = 17

Table 2. continued.

	HMHF	CMCF	HMCF	CMHF
vv bur avg				
succ.	20.21	27.76	23.04	22.62
	41.97%	32.96%	34.19%	43.84%
	(8.48)	(9.15)	(7.88)	(9.92)
	n = 74	n = 76	n = 82	n = 46
unsucc.	19.48	24.24	27.17	26.78
	(5.87)	(4.55)	(5.84)	(11.00)
	30.14%	18.76%	21.51%	41.07%
	n = 15	n = 15	n = 8	n = 39
vv bur var				
succ.	7.63	10.06	8.23	8.59
	(4.16)	(4.30)	(3.44)	(3.35)
	54.49%	42.73%	41.87%	38.93%
	n = 74	n = 76	n = 82	n = 46
unsucc.	6.97	9.58	10.31	10.69
	(3.00)	(4.35)	(3.09)	(5.66)
	43.04%	45.33%	29.94%	52.95%
	n = 15	n = 15	n = 8	n = 39
vv axs				
succ.	317.94	326.74	372.51	292.28
	(90.98)	(107.93)	(84.50)	(105.59)
	28.62%	33.03%	22.68%	36.13%
	n = 74	n = 76	n = 82	n = 46
unsucc.	280.53	363.40	306.22	247.25
	(79.46)	(81.59)	(99.09)	(102.56)
	28.32%	22.45%	32.36%	41.48%
	n = 15	n = 15	n = 8	n = 39
dv pol dis*				
succ.	11.18	12.62	11.57	11.23
	(1.91)	(2.84)	(2.12)	(2.50)
	-----	-----	-----	-----
	n = 70	n = 64	n = 81	n = 29
unsucc.	9.87	9.07	10.68	10.93
	(3.43)	(4.24)	(2.12)	(2.54)
	-----	-----	-----	-----
	n = 15	n = 15	n = 5	n = 16

Table 2. continued.

	HMHF	CMCF	HMCF	CMHF
dv pol ang°				
succ.	268.35	271.32	268.20	270.26
	(7.98)	(7.69)	(6.35)	(9.24)
	n = 70	n = 64	n = 81	n = 29
unsucc.	269.50	273.25	284.40	268.56
	(13.29)	(9.31)	(13.32)	(18.35)
	n = 15	n = 15	n = 5	n = 16
vv pol dis°				
succ.	1.69	3.68	2.58	2.41
	(1.74)	(2.88)	(1.74)	(2.16)
	n = 74	n = 76	n = 82	n = 53
unsucc.	2.46	3.01	3.90	4.96
	(2.29)	(2.37)	(2.97)	(3.09)
	93.09%	78.74%	76.15%	62.30%
	n = 15	n = 15	n = 8	n = 39
vv pol ang°				
succ.	180.15	244.26	220.13	166.97
	(102.98)	(76.60)	(84.39)	(98.26)
	n = 64	n = 74	n = 79	n = 45
unsucc.	185.46	160.59	248.38	215.57
	(106.41)	(94.17)	(76.27)	(76.21)
	n = 13	n = 14	n = 8	n = 38
dv rot°				
succ.	0.82	0.34	0.07	0.12
	(1.81)	(3.12)	(1.89)	(3.19)
	n = 70	n = 64	n = 81	n = 29
unsucc.	0.83	0.58	1.90	1.03
	(1.99)	(1.93)	(3.78)	(4.25)
	n = 15	n = 15	n = 5	n = 16

Table 2. continued.

	HMHF	CMCF	HMCF	CMHF
vv rot*				
succ.	0.14 (2.03) ----- n = 74	1.05 (3.34) ----- n = 76	0.30 (1.67) ----- n = 82	0.10 (4.33) ----- n = 53
unsucc.	0.86 (1.74) ----- n = 15	0.00 (2.22) ----- n = 15	0.38 (3.69) ----- n = 8	0.85 (5.27) ----- n = 39

* Since the orientation measurements represented direction and not size, coefficients of variation were inappropriate for comparing variability. Instead, standard deviations may be compared directly among and within the crosses.

Genitalic and body size characters: descriptive statistics.

Pairwise comparisons revealed significant inter-correlations among the genitalic variables in this study (Table 3). In general, two types of association were common:

(1) Variables measured on the same valves were often significantly correlated (e.g. the medial axis length between the dorsal valves and bursa was significantly correlated with the penetration of the dorsal valves).

(2) Similar classes of genitalic variables were significantly correlated (e.g. dorsal and ventral valve penetrations were strongly associated).

In addition to correlations among the genitalic variables, the difference in male and female sizes was associated with many of the measures of genitalic fit. Of the fifteen measures of genitalic incompatibility recorded, male and female size difference was correlated ($p <= 0.05$) with ten of these measures. Although several other variables had a greater number of significant correlations (e.g. the unoccupied bursal area was significantly correlated with 13 genitalic variables), many of these statistical associations were strongly influenced by body size differences (reported later).

An inspection of means, standard deviations, and coefficients of variation for each variable (Tables 1 and 2) suggested several critical features of genitalic fit in B. humphreysii which are discussed more fully later:

(1) Extreme male and female size differences as well as a large distance between aedeagal apices and the spermathecal duct opening (i.e. shallow penetration) characterized the less

Table 3. Pearson correlation coefficients for log transformed data. (Correlations are listed above, levels of significance below)

	dv pen	vv pen	unc bur	dv axs	vv axs	dv bur avg	dv bur var	vv bur avg	vv bur var	dv pol dis	dv pol ang	vv pol dis	vv pol ang	dv rot	vv rot
size	-.24	-.16	-.11	-.24	-.17	-.19	-.22	.03	-.05	.03	-.03	-.04	-.12	.17	.12
diff	.00	.00	.03	.00	.00	.00	.00	.52	.35	.59	.62	.44	.03	.00	.02
dv pen		.67	.55	.62	.59	.37	.31	.18	-.06	-.07	.14	-.26	.11	-.18	-.20
		.00	.00	.00	.00	.00	.00	.00	.26	.26	.02	.00	.05	.00	.00
vv pen			.51	.21	.66	.11	.02	.32	.15	.11	.10	-.07	.17	-.01	-.10
			.00	.00	.00	.06	.77	.00	.00	.06	.07	.17	.00	.86	.05
unc bur				.40	.30	.34	.38	.13	-.04	.26	.12	-.21	.00	-.20	-.38
				.00	.00	.00	.00	.01	.45	.00	.05	.00	.97	.00	.00
dv axs					.41	.45	.47	-.11	-.03	-.14	.11	-.21	-.03	-.22	-.32
					.00	.00	.00	.07	.66	.02	.06	.00	.61	.00	.00
vv axs						.02	.06	.24	.20	.13	.18	-.21	.09	-.06	-.22
						.79	.28	.00	.00	.03	.00	.00	.11	.29	.00
dv bur avg							.66	.07	.02	-.09	-.08	-.02	.01	-.22	-.13
							.00	.24	.77	.11	.19	.70	.86	.00	.02
dv bur var								-.00	-.01	-.01	-.09	.00	-.01	-.16	-.17
								.97	.87	.81	.14	.98	.83	.01	.00
vv bur avg									.59	.16	-.02	.31	.15	.14	.13
									.00	.01	.76	.00	.00	.02	.01
vv bur var										.06	-.10	.29	.13	.10	.23
										.31	.09	.00	.02	.08	.00

Table 3. continued

	dv pen	vv pen	unc bur	dv axs	vv axs	dv bur avg	dv bur var	vv bur avg	vv bur var	dv pol dis	dv pol ang	vv pol dis	vv pol ang	dv rot	vv rot
dv											.17	.32	.15	-.00	-.11
pol											.00	.00	.01	.97	.07
dis															
dv												-.22	.11	-.15	-.32
pol												.00	.08	.01	.00
ang															
vv													.48	.15	.29
pol													.00	.01	.00
dis															
vv														.04	.15
pol														.48	.01
ang															
dv															.68
rot															.00

successful CMHF cross compared to the other three crosses (Table 1). Extreme body size differences in the CMHF cross probably altered the ability of males to reach around the female abdomen and penetrate the bursa to an adequate depth (Appendix I). These data suggested that large differences in male and female body size may have been the proximal cause of the shallow penetration and lower spermatophore transfer rate in the CMHF cross compared to the more similar body sizes, deeper penetrations, and greater success of the other three crosses.

(2) Although extreme size differences in the CMHF cross may have lowered spermatophore transfer success with respect to the other three crosses, size differences were not significantly associated with transfer success within the CMHF cross (Table 4, MANOVA, $p = 0.1694$; also see Table 2). Instead, the 58% success rate in this cross may have represented a threshold associated with shallow penetration of the bursa by extremely small males. If the majority of copulating pairs were near the tolerance limit of large differences in male and female body size (i.e. penetration allowing spermatophore transfer was about as likely as inadequate penetration), then no association between size differences and spermatophore transfer would be expected. (In a statistical sense, the regression line between body size differences and transfer frequencies would be flat. Although not specifically tested, if success rates had dropped quickly from 58% to 0% with slightly greater size differences and shallower penetrations, a threshold effect might have been confirmed.)

Table 4. MANOVA (listed first) and MANCOVA (listed second) of genitalic and body size variables with respect to spermatophore transfer. (Significance levels are reported for each variable.)

	HMHF		CMCF		HMCF		CMHF	
size diff	.0773	---	.0433	---	.0800	---	.1694	---
dv pen	.5725	.2832	.6727	.7828	.0100	>.03	.6311	.4046
vv pen	.2375	.0794	.7557	.7642	.2530	.3422	.0508	>.02
unc bur	.6030	.8693	.0584	.1297	.0550	>.03	.0414	>.03
dv axs	.6677	.9766	.0265	>.04	.2673	.3718	.5494	.8809
vv axs	.6149	.7311	.5154	.6840	.1248	.1991	.7147	.7611
dv bur avg	.9331	.8381	.0249	>.07	.4999	.6143	.6240	.4378
dv bur var	.6707	.6496	.0234	>.11	.7776	.8116	.6104	.2741
vv bur avg	.7317	.5668	.4626	.2895	.2197	.2191	.0013	.0037
vv bur var	.7512	.6284	.6714	.9426	.2747	.1571	.0480	>.06
dv pol dis	.1001	.1056	.0001	.0004	.3787	.4770	.5496	.4244
dv pol ang	.0076	.0100	.2455	.4091	.0001	.0001	.0535	>.03
vv pol dis	.0299	>.06	.8325	.7230	.0100	>.01	.0012	.0011
vv pol ang	.1139	.2210	.8441	.8626	.9499	.9876	.2896	.2830
dv rot	.6973	.7561	.2441	.2617	.1005	.1852	.3263	.5777
vv rot	.4861	.6518	.2283	.2066	.0369	>.05	.0062	.0100

(MANOVA variables were considered significant if p <= .03. MANCOVA variables were significant if p <= 0.01. Variables significant in the MANCOVA were considered size-free estimators of insemination success.)

(3) Within each of the highly successful HMHF, CMCF, and HMCF crosses, the success of spermatophore transfer was significantly associated with differences in male and female body sizes (Table 4, MANOVA: HMHF, $p = 0.0773$; CMCF, $p = 0.0433$; HMCF, $p = 0.0800$; also see Table 2; MANOVA results are discussed extensively later in this chapter). In the intra-racial crosses, relatively smaller males and larger females were successful, but in the HMCF cross, relatively larger males and smaller females were successful. In the HMCF cross, more similar male and female sizes may have enabled males to reach around the female and penetrate the bursa to an adequate depth (Appendix I), thus overcoming the effects of genitalic incompatibility anticipated by Cohn and Cantrall (1974; discussed later). However, in the intra-racial crosses, females may have preferred to mate with smaller males, perhaps as a consequence of sexual selection (Eberhard, 1985; discussed later).

(4) Within and among crosses (Table 2), the average values for most genitalic variables were remarkably similar for successful and unsuccessful copulating pairs. In addition, there was no consistently higher variation associated with unsuccessful copulating pairs when compared to successful pairs. [Greater variation in genitalic fit was expected among unsuccessful mated pairs (see chapter one).] Medial axis measurements showed especially high coefficients of variation in both successful and unsuccessful groups. The medial axis data indicated a great deal of variation in morphological compatibility was tolerated in the intra-racial and inter-

racial crosses. In addition, multivariate analyses (reported later) suggested that the effects of genitalic fit on spermatophore transfer were largely explained by differences in male and female body sizes.

Observations of the histological sections.

Several features of genitalic incompatibility were apparent from a careful examination of the histological slides and were later confirmed by multivariate analysis.

In all four crosses:

(1) The dorsal and ventral valves penetrated the bursa as a unit, the ventral valves penetrated more deeply than the dorsal valves, and the dorsal and ventral valves rotated about a common aedeagal axis within the bursa.

In the HMHF, CMCF and HMCF crosses (figures 9, 10 and 11, respectively):

(2) The ventral valves were located very close to the center of the bursal reference system in a position above the dorsal valves. Since the aedeagus was twisted 180 degrees during insertion (figure 1, and see chapter 2), the dorsal location of the ventral valves within the bursa was considered normal.

(3) The dorsal valves were located ventral to the center of the bursa in a position below the ventral valves. Given the twisting insertion of the aedeagus, the ventral location of the dorsal valves was normal.

(4) Both the dorsal and ventral valves penetrated deeply, fitting closely to the bursal wall, and with relatively parallel curvature.

Figure 9. Serial cross-sections of the HMHF cross (B. h. humphreysii male X B. h. humphreysii female, specimen # 002).

A. 15% of the length of the bursa

B. 30% of the length of the bursa

C. 45% of the length of the bursa

Symbols used in the figure:

BR - bursa

DV -dorsal valves

VV -ventral valves

Figure 10. Serial cross-sections of the CMCF cross (B. h. cochisei male X B. h. cochisei female, specimen # 103).

A. 15% of the length of the bursa

B. 30% of the length of the bursa

C. 45% of the length of the bursa

Symbols used in the figure:

BR - bursa

DV -dorsal valves

VV -ventral valves

Figure 11. Serial cross-sections of the HMCF cross (B. h. humphreysii male X B. h. cochisei female, specimen # 114).

A. 15% of the length of the bursa

B. 30% of the length of the bursa

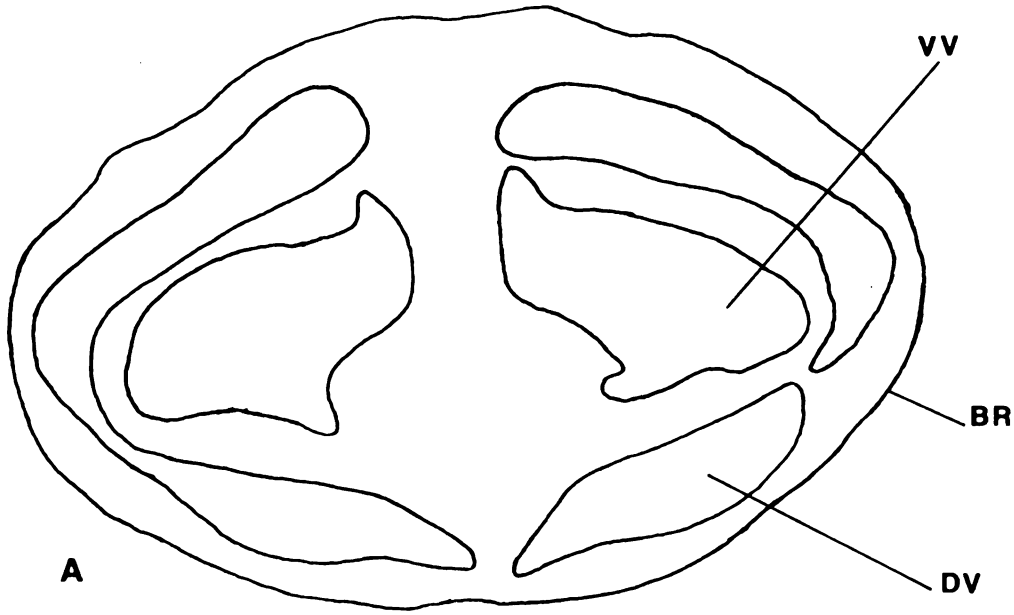
C. 45% of the length of the bursa

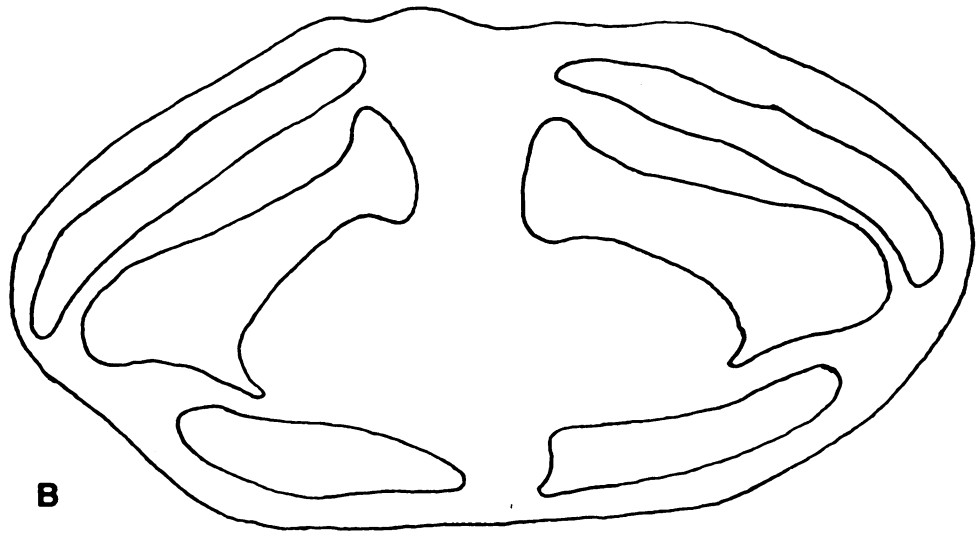
Symbols used in the figure:

BR - bursa

DV -dorsal valves

VV -ventral valves


VV
BR
DV
A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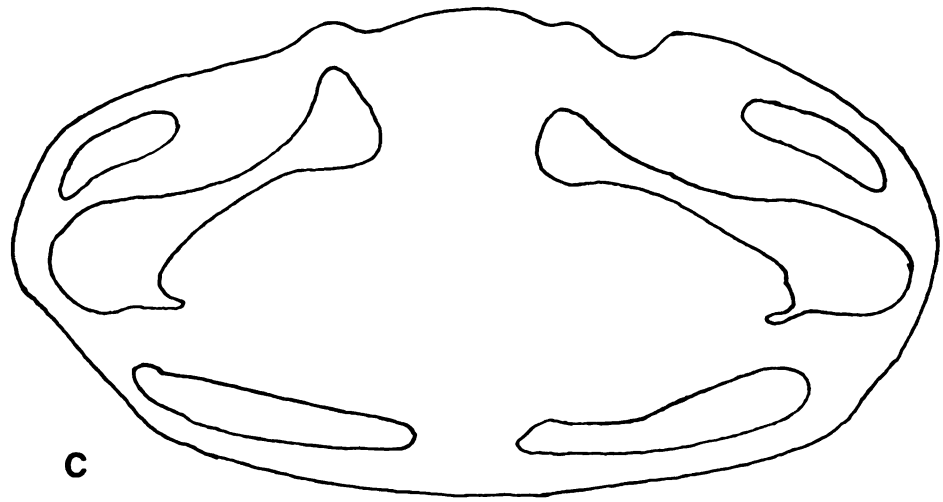
C

Figure 12. Serial cross-sections of the CMHF cross (B. h. cochisei male X B. h. humphreysii female, specimen # 146). Note that the right ventral aedeagal valve is visible outside of the bursa. In this case, penetration completely failed.

A. 15% of the length of the bursa

B. 30% of the length of the bursa

C. 45% of the length of the bursa

Symbols used in the figure:

BR - bursa

VV -ventral valves

Figure 13. Serial cross-sections of the CMHF cross (B. h. cochisei male X B. h. humphreysii female, specimen # 166). Note that only the apical portions of the ventral valves have penetrated the bursa at these depths. The dorsal valves are completely absent.

A. 15% of the length of the bursa

B. 30% of the length of the bursa

C. 45% of the length of the bursa
Symbols used in the figure:

BR - bursa

VV -ventral valves

In the HMCF cross (figure 11):

(5) The aedeagus penetrated deeply and fit snugly against the bursa. In particular, basal portions of the aedeagus requiring deep penetration appeared in many of the cross-sections, a condition not observed in the other crosses. Bursal pleating was also much more stretched out by deep penetration than in the other crosses. Bursal stretching may have caused a better correspondence in male and female curvature than expected by Cohn and Cantrall (1974) based on genital morphologies. In addition, deep penetration may have overcome the effects of spermatophore deflection anticipated by these authors (discussed in chapter 2).

In the CMHF cross (figures 12 and 13):

(6) The penetration of the aedeagus was shallow, and in some cases the aedeagus was entirely absent from the histological sections of the bursa (figure 12). On occasion, only the ventral valves penetrated the bursa, while the dorsal valves remained lodged in the genital chamber just outside the bursa (figure 13). Shallow penetration may have compounded the effects of mismatched genitalia (Cohn and Cantrall, 1974), leading to a low frequency of spermatophore transfer.

These histological observations indicated a great deal about genitalic fit in Barytettix. Similar procedures may be useful with other organisms. In addition, histological observations can be compared to statistical analyses to arrive at a better understanding of genitalic fit.

The functional grouping of genitalic variables.

Cluster analysis

Despite the clearly founded lock-and-key predictions of Cohn and Cantrall, spermatophore transfer frequencies among crosses of B. humphreysii showed unexpected asymmetry in the inter-racial crosses. The CMHF cross was markedly less succesful than the intra-racial crosses as predicted, but the HMCF cross was at least as successful as the intra-racial crosses in the transfer of spermatophore material.

Although the high transfer frequency in the HMCF cross was contrary to the predictions of Cohn and Cantrall, this result does not directly refute the lock-and-key hypothesis. Rather, the unexpected success of the the HMCF cross suggested that some aspects of genitalic fit in the HMCF cross should be shared in common with the intra-racial crosses. In addition, the low frequency of spermatophore transfer in the CMHF cross indicated genitalic fit was either poorer than the other crosses, or was perhaps fundamentally different.

According to the lock-and-key hypothesis, high frequencies of spermatophore transfer among the HMCF, HMHF, and CMCF crosses should be due to a similar fit of the genitalia. Extending this argument further, if these three crosses have functionally equivalent genitalia, then they should also covary in their estimates of genitalic fit in ways that can be explained by similar groupings of variables. Clusters were calculated by PROC VARCLUS (SAS Institute, 1985), and compared among crosses to determine if any clusters were shared in common among the successful crosses as predicted by the lock-and-key hypothesis. PROC VARCLUS was also used to group genitalic variables in the CMHF cross. Since the CMHF cross was less successful than the other crosses, a poorer

genitalic fit might be indicated by a dissociation of the clusters found in the HMCF, HMHF, and CMCF crosses.

Table 5 shows a striking correspondence among the clusters in the successful HMCF, HMHF, and CMCF crosses. With well over 80% of the variation explained in each cross, eleven cluster groupings were identical among the HMCF, HMHF, and CMCF crosses, but quite different for the CMHF cross. These results suggest the genitalia in the HMCF, HMHF, and CMCF crosses fit together similarly to yield high transfer frequencies, but the genitalia in the CMHF cross fit together differently to lower transfer frequency. Specific interpretations of the clusters were derived after a thorough inspection of the slides. (The sequence of cluster splitting was not identical for any of the crosses.)

Clusters in common among the four crosses

In all four crosses, the depths of penetration of the dorsal and ventral valves formed a single cluster (i.e. the distances between the spermathecal duct opening and the apex of each set of dorsal and ventral valves were clustered together). In addition, the relative rotations of the dorsal and ventral valves within the bursa formed a single cluster. The first finding confirmed that the aedeagal valves penetrated the bursa as a unit. Although this observation was obvious to the biologist, it is statistically a non-trivial result. The second finding verified the dorsal and ventral valves rotated in common about the long axis of the aedeagus. This common rotation was intuitively clear in the slides.

Table 5. Clusters of genitalic and body size variables for the intra-racial and inter-racial crosses of B. humphreysii. (Values reported are 1 - R^2 ratios*.)

clustered variables	HMHF	CMCF	HMCF	CMHF	clustered variables
vv bur avg	0.15	0.32	0.34	----	
vv bur var	0.20	0.30	0.23	0.00	vv bur var
dv bur avg	0.17	0.29	0.20	0.25	dv bur avg
dv bur var	0.18	0.33	0.20	0.22	dv bur var
				0.48	dv axs
dv rot	0.24	0.13	0.23	0.21	dv rot
vv rot	0.27	0.12	0.26	0.21	vv rot
dv pen	0.12	0.15	0.09	0.10	dv pen
vv pen	0.16	0.15	0.08	0.08	vv pen
vv pol dis	0.24	0.26	0.26	0.00	vv pol dis
vv pol ang	0.22	0.25	0.23	0.00	vv pol ang
size diff	0.00	0.00	0.00	0.26	size diff
	----	----	----	0.26	vv bur avg

Table 5. continued

dv pol dis	0.00	0.00	0.00	0.00	dv pol dis
dv pol ang	0.00	0.00	0.00	0.00	dv pol ang
unc bur	0.00	0.00	0.00	0.00	unc bur
vv axs	0.00	0.00	0.00	0.00	vv axs
dv axs	0.00	0.00	0.00	____	

*

The ratio of one minus the squared correlation of a variable with its own cluster component to one minus the next highest squared correlation of that variable with another cluster.
This value is low if the clusters are well separated.

Horizontal lines separate cluster groups. Note that the HMCF, HMHF, and CMCF crosses share identical clusters.

The HMCF, HMHF, and CMCF crosses

Inspection of the slides suggested a stable location of the ventral valves within the bursa of the HMCF, HMHF, and CMCF crosses. The center of the ventral valves coincided closely with the geometric center of the bursa, varying little about this point (Table 1 means and standard deviations of the polar coordinate distance in microns were: HMHF, 1.86 and 1.82; CMCF, 3.60 and 2.80; HMCF, 2.71 and 1.91). [The polar coordinate angle was highly variable in these three crosses (Table 1), indicating that the ventral valves occurred within a broad range of degrees away from the positive x-axis of the bursal reference system. However, given the extreme close proximity of the ventral valves to the bursal center, these angular fluctuations probably had relatively little impact on the alignment of the ventral valves.]

Ventral valve stability was also suggested by the identical cluster of polar coordinates (distance and angle) shared among these three successful crosses (Table 5). The consistent appearance of this cluster in each cross indicated the spatial location of the ventral valves may have influenced spermatophore transfer. For example, a stable central location of the ventral valves could have optimally guided the spermatophore tube. In the CMCF cross, the closed medial margins of ventral valves (located in the center of the bursa) might have deflected the spermatophore toward the spermathecal opening in the anterior center of the bursa (figures 4 and 5a). In the HMHF cross, the open medial margins of the centrally located valves could have guided the spermatophore dorsad toward the antero-dorsal opening of the spermatheca (figures 2 and 5b). [Cohn and Cantrall (1974) suggested that ventral valve morphology closely matches the position of the spermathecal opening in intra-racial crosses.]

In the HMCF cross, deep penetration of the aedeagus (comparable to the intra-racial crosses, especially the CMCF cross, Tables 1 and 2) brought the genital openings close together. In addition, deep penetration of the ventral valves along the central axis of the bursa may have placed the spermatophore tube close to the opening of the centrally located spermathecal duct (figure 5e). By decreasing the distance the spermatophore tube had to travel, the deep ventral valve location may have overcome the error in spermatophore transfer due to deflection (discussed in chapter two). Deeper penetration was likely a consequence of large male body size and will be discussed later.

Less stability in the dorsal valve location was indicated by the occurrence of the dorsal vavle polar coordinates in separate clusters (Table 5). Although the dorsal valves were located at consistent distances ventral to the bursal center (Table 1 means and standard deviations in microns were: HMHF, 10.97 and 2.29; CMCF, 12.12 and 3.30; HMCF, 11.52 and 2.12), they behaved erratically within this region. In particular, the polar coordinate angle was unstable. The dorsal valves were found 244 to 302 degrees away from the positive x-axis in the HMCF cross, 253 to 301 degrees in the HMHF cross, and 256 to 290 degrees in the CMCF cross. (These ranges were 58, 48, and 34 degrees, respectively).

The average radial distance and the variation in this distance were also grouped together in separate dorsal and ventral valve clusters. These repeated clusters indicated a closely curving fit between the aedeagus and the bursa in the HMCF, HMHF, and CMCF crosses. A strong complementarity in genitalic curvature was anticipated in the intra-racial crosses (Cohn and Cantrall, 1974), and may have stabilized movements of the genitalia, enhancing spermatophore transfer. The

curving fit between the aedeagus and bursa in the HMCF cross was probably due more to the stretching of the bursa than the morphological complementarity of the genitalia. The large males in this cross penetrated deeply (Tables 1 and 2), stretching the bursa slightly (figure 11) and improving the curvature between male and female genitalia. This stretching may have offset the morphological incompatibility anticipated by Cohn and Cantrall (1974).

In summary, cluster analysis in all four crosses indicated that the dorsal and ventral valves penetrated as a unit, and that these valves rotated in common about the long axis of the aedeagus. In addition, several clusters were shared among the highly successful HMCF, HMHF, and CMCF crosses:

(1) The common cluster of ventral polar coordinates reflected a stable location of the ventral valves within the bursa. The dissociated dorsal valve coordinates indicated a less stable location of the dorsal valves in the bursa. Inspection of the slides also supported this finding. Either one or both of these factors may have influenced spermatophore transfer.

(2) The average radial distance and the variation in this distance were clustered together in both sets of aedeagal valves. This indicated the dorsal and ventral valves fit closely and relatively parallel to the bursal wall in the intra-racial crosses. In the HMCF cross, this closely curving fit was probably due to a slight stretching of the bursa brought on by the deep penetration of the aedeagus. Visual inspection of the histological slides supported these findings.

The CMHF cross

In the CMHF cross, males were much smaller than females and often had difficulty mounting and penetrating the bursa (Appendix I). Inspection of the slides showed the aedeagus only shallowly penetrated the bursa during copulation. Probably as a consequence of small male size (discussed later), the dorsal valves were often absent from the slides (69 of 114 total sections missed dorsal valves; see figures 12 and 13). A lack of dorsal valves within a section was seldom encountered in the three successful crosses (HMCF and CMCF crosses missed dorsal valves in 3 of 93 sections, the HMHF cross in 8 of 93 sections). The absence of dorsal valves, even at shallow depths of the bursa, must have contributed to the low spermatophore transfer success in the CMHF cross. Without the dorsal valves, it is doubtful the ventral valves could have adequately guided the spermatophore tube to the spermatheca. Instead, the spermatophores may have been deflected into the bursal wall (figure 5f) or ejaculated into the genital cavity (Pickford and Gillot, 1971). In addition, the shallow aedeagal penetration increased the distance the spermatophore tube had to travel to reach the spermathecal opening (see Tables 1 and 2), probably inhibiting successful transfer.

When the aedeagus penetrated the bursa, the dorsal valve location was highly unstable. This was evident in the slides and was suggested by the statistical dissociation of the polar coordinates into separate single-member clusters. Comparisons of the four crosses indicated the dorsal valves were most unpredictably located in the CMHF cross. The polar coordinate angle was especially unstable, occuring 226 to 295 degrees away from the postive x-axis of the bursal reference system (a range of 69 total degrees, the greatest of all four crosses). There

were several instances when the dorsal valves were found dorsal to the center of the bursa in the range of 0 to 180 degrees from the postive x-axis of the bursal reference system. This highly atypical condition never occurred in the HMCF, HMHF, or CMCF crosses, and was indicative of the poorly fitting genitalia in this cross.

Also in the CMHF cross, the measurements of ventral valve curvature no longer clustered together as they did in the three successful crosses. Instead, the average radial distance occured in a common cluster with body size differences, while the variation in this distance clustered alone. The ventral valve polar coordinates also dissociated into single-member clusters. Dissociation of the curvature and polar coordinate clusters suggested the location of the ventral valves within the bursa was highly unstable compared to the three successful crosses. Along with the shallow penetration of the dorsal valves, this unstable location of the ventral valves may have contributed to the low frequency of spermatophore transfer in the CMHF cross.

The single cluster of curvature variables associated with the dorsal valves was somewhat surprising in the CMHF cross. As mentioned above, the dorsal valves were erratically located and sometimes absent from entire sections of the copulating genitalia. It was difficult to believe the curving fit of the dorsal valves and bursa improved the compatibility of the CMHF cross, when the spermatophore transfer frequency was very low compared to the successful crosses. As an alternative explanation, covariation in these measures may have been artifactual. The absence of the dorsal valves from many sections may have introduced sampling error into the curvature covariation, resulting in a misleadingly cluster of these dorsal valve measures.

Cluster analysis indicated the genitalia in the CMHF cross fit together and function in a fundamentally different way than the successful HMCF, HMHF, and CMCF crosses. Although the penetration and relative rotation of the dorsal and ventral valves were shared with the successful crosses, many of the remaining variables were clustered together differently. In summary:

(1) The instability of the ventral valves was indicated by the absence of a curvature cluster (the average radial distance and variation in these distances), and the absence of a polar coordinate cluster (the polar coordinate distance and angle) when compared to the presence of these clusters in each of the relatively successful HMHF, CMCF, and HMCF crosses.

(2) The instability of the dorsal valves was especially apparent from visual inspection of the slides. The dorsal valves were frequently absent from sections of the bursa, indicating a shallow aedeagal penetration probably due to small male size. When the dorsal valves were present in the bursal sections, their location occasionally strayed above the geometric center of the bursa, an atypical location. The shallow aedeagal penetration in the CMHF cross undoubtedly increased the distance over which the spermatophore tube had to travel to reach the spermathecal opening, and may have contributed to the low frequency of spermatophore transfer in this cross.

The influence of male and female body size on genitalic fit.

Discriminant analysis

Multivariate analysis of covariance

Body size differences influenced the male's ability to penetrate the female bursa in all four crosses (Appendix I), and were also statistically correlated with many of the genitalic variables (this chapter). To better understand how genitalic fit influenced spermatophore transfer, it was necessary to control the confounding effects of body size differences on the incompatibility measures. Discriminant analysis and multivariate analysis of covariance (MANCOVA) provided two methods for accomplishing this task.

Discriminant analysis (PROC STEPDISC; SAS Institute, 1985) was used to reduce the full model of genitalic variables (see chapter three) through an iterative backward elimination process. At each step, the one variable contributing least to the discrimination of spermatophore transfer success at the 0.01 level of significance was removed. Body size differences were specified to always remain in the discriminant model. Because body size differences acted as covariates on the non-included variables, only estimates of genitalic fit that were relatively unassociated with body size differences were retained as predictors of spermatophore transfer. MANCOVA tests (PROC GLM) were performed on each cross with a similar purpose. By using only male and female size differences as a covariate, the effects of size on each genitalic variable were removed by regression. Only those variables relatively independent of body size differences were likely to show a significant association with spermatophore transfer success. Genitalic variables were considered significantly associated with spermatophore transfer in

the MANOVA at the 0.03 level of significance. In the MANCOVA, genitalic variables were associated with spermatophore transfer, independent of body size differences, if p <= 0.01.

The following MANCOVA model was used to determine the differences in genitalic fit between successful and unsuccessful copulating individuals with respect to the classification ("treatment") variable, spermatophore transfer (see Table 1 for abbreviations):

(Spermatophore transfer success) = { -b (body size difference)} {(dv to sp) + (vv to sp) + (unc bur) + (dv axs) + (vv axs) + (dv bur avg) + (dv bur var) + (vv bur avg) + (vv bur var) + (dv pol dis) + (dv pol ang) + (vv pol dis) + (vv pol ang) + (dv rot) + (vv rot)}

where b is the regression coefficient for body size difference. Thus, the effect of body size difference was subtracted from each dependent genitalic variable.

In the MANOVA model, the covariate (body size difference) appeared instead as an additional dependent variable:

(Spermatophore transfer success) = (body size difference) + (dv to sp) + (vv to sp) + (unc bur) + (dv axs) + (vv axs) + (dv bur avg) + (dv bur var) + (vv bur avg) + (vv bur var) + (dv pol dis) + (dv pol ang) + (vv pol dis) + (vv pol ang) + (dv rot) + (vv rot).

Intra-racial crosses: HMHF and CMCF

According to cluster analyses and based on inspection of the slides, the location of the dorsal valves in the intra-racial crosses was considered less stable than the ventral valves. This instability was underscored by the discriminant analysis. The final discriminant

Table 6. Discriminant analysis of genitalic and body size variables with respect to spermatophore transfer. [The final discriminant model in each cross included the tabulated variable (which remained in the model at the 0.01 level of significance or less), and the difference between male and female body sizes (which was forced to remain at all stages of the backward elimination process). The tabulated variables were considered size-free estimators of spermatophore transfer.]

HMHF	dorsal valve polar coordinate angle	p = 0.0100
CMCF	dorsal valve polar coordinate distance	p = 0.0001
HMCF	dorsal valve polar coordinate angle	p = 0.0001
	ventral valve penetration	p = 0.0068
CMHF	ventral valve average radial distance	p = 0.0015
	ventral valve rotation	p = 0.0075

models for both crosses included a polar coordinate measure of genitalic fit at the 0.01 significance level or less (Table 6), along with the difference in male and female body size forced to remain in the model.

In the HMHF cross, the dorsal valve polar coordinate angle ($p = 0.01$) was an important part of the size-controlled discriminant model. As a discriminator of spermatophore transfer success, the polar coordinate angle was less variable in the successful group than the unsuccessful group (Table 2 means and standard deviations in degrees were: successful, 268.35 and 7.98; unsuccessful, 269.50 and 13.29). Additionally in the model, females were much larger than males (i.e. size differences were greater) in the successful group than the unsuccessful group (means and standard deviations in milligrams were: successful, 594.62 and 195.98; unsuccessful, 490.00 and 161.82).

In the CMCF cross, the polar coordinate distance ($p = 0.0001$) was a significant discriminator in the size-controlled model. The dorsal valves were more distant from the center of the bursa in the successful group than the unsuccessful group (Table 2 means and standard deviations in microns were: successful, 12.62 and 2.84; unsuccessful, 9.07 and 4.24). Females were also much larger than males (i.e. size differences were greater) in the successful group than in the unsuccessful group (successful, 498.15 and 163.26; unsuccessful, 407.50 and 55.94).

In the unsuccessful HMHF group, greater variability in the dorsal valve angle represented variation in the location and orientation of these valves. Such variation probably countered the stability of the ventral valves, producing inconsistent spermatophore trajectories and inhibiting successful transfer. In the successful group, less variation in dorsal valve location probably helped the ventral valves maintain the antero-dorsal trajectory of the spermatophore tube required for

spermatophore transfer (Cohn and Cantrall, 1974; also see chapter two). In the unsuccessful CMCF group, dorsal valves close to the bursal center may have deflected the spermatophore in undesirable ways. Since the spermatheca opened at the anterior center of the bursa, dorsal valves close to the bursal center may have obstructed or deflected spermatophores from a central trajectory most likely to reach the spermatheca [as determined by the ventral valves (Cohn and Cantrall, 1974)]. In the successful group, dorsal valves located farther from the bursal center probably left the central path of the spermatophore tube free from obstruction.

It is unclear how an increase in male size relative to female size inhibited spermatophore transfer in both intra-racial crosses. Hypothetically, larger males should have penetrated more deeply than smaller males, bringing the genital openings closer together and increasing the likelihood of spermatophore transfer. But contrary to this expectation, males in the intra-racial crosses were more similar to females in body size in the unsuccessful groups, and much smaller than females in the successful groups (Table 2). As a consequence, the distance between the genital openings was less (i.e. penetration was deeper) in the unsuccessful groups than in the successful groups. [Table 2 means and standard deviations for the distance between the spermathecal duct opening and the apex of the dorsal valves in microns were: HMHF successful, 98.65 and 50.97; HMHF unsuccessful, 87.00 and 55.03; CMCF successful, 230.56 and 89.83; CMCF unsuccessful, 168.75 and 91.60. Means and standard deviations for the distance between the spermathecal opening and the apex of the ventral valves in microns were: HMHF successful, 55.38 and 53.58; HMHF unsuccessful, 30.00 and 27.77;

CMCF successful, 72.22 and 58.68; CMCF unsuccessful, 26.25 and 27.97.]

Although larger males penetrated more deeply than relatively smaller males, if body size differences were used by copulating females to chose among males before ejaculation (Eberhard, 1985), then the depth of penetration may have had no necessary bearing on spermatophore transfer. Although conjectural, females may have rejected intra-racial males close to their own size by refusing to engage in mutual courtship behaviors necessary to stimulate male ejaculation. The basis of a size choice within intra-racial populations might be related to a proportionality criterion. Females may have judged males such that an average or constant population sexual dimorphism was maintained at the level of the individual pair. Alternatively, females may have choosen among characters correlated with body size such as aedeagal dimensions, or the tactile cues associated with aedeagal penetration. A female preference function based on the size-dependent rejection of males or upon size-correlated male characters must be demonstrated to validate this assumption (see chapter 6).

The foregoing discriminant analyses suggested the location of the dorsal valves was the only predictor of spermatophore transfer unbiased by size in the intra-racial crosses. Although cluster analyses revealed additional variables relevant to genitalic fit (distance between the genital openings, rotation of the aedeagal valves, ventral valve polar coordinates, and the curving fit of the genitalia), the absence of these variables from the final discriminant model suggested they were adequately explained by body size differences. The ability of body size differences to account for most of the genitalic variables was corroborated by MANOVA and MANCOVA tests.

Table 4 (MANOVA only) shows that several genitalic variables were initially associated with spermatophore transfer in the intra-racial crosses. In the CMCF cross, estimates of dorsal valve location and curvature were initially associated with spermatophore transfer, while in the HMHF cross, measures of dorsal and ventral valve locations were important. When body size differences were used as covariates to adjust the other genitalic variables (MANCOVA), only the dorsal valve distance ($p = 0.0004$) was significantly associated with spermatophore transfer in the CMCF cross, and only the dorsal valve angle ($p = 0.01$) was important in the HMHF cross. Since these were the same dorsal valve variables that predicted spermatophore transfer success in the discriminant models of each cross, the combined results of the discriminant and MANOVA/MANCOVA tests suggested that:

(1) Male and female body size differences explained the effect of all estimates of genitalic fit on spermatophore transfer, except the location of the dorsal valves.

(2) Male and female size differences, along with the relatively size-independent location of the dorsal valves, may be primarily responsible for the high frequency of successful spermatophore transfer in the intra-racial crosses.

The HMCF cross

In the HMCF cross, the size-controlled discriminant model (Table 6) showed that spermatophore transfer was significantly associated with the distance between the opeining of the spermathecal duct and the apex of the ventral valves ($p = 0.0068$), and the dorsal valve polar coordinate angle ($p = 0.0001$). The distance between the spermathecal opening and the ventral valve apex was less (i.e. penetration was deeper) in the

successful group than the unsuccessful group [Table 2 means and standard deviations in microns were: successful, 87.32 and 56.76; unsuccessful, 140.00 and 78.30. These values were not significantly different in the MANOVA ($p = 0.2530$, Table 4).] Also, the polar coordinate angle was less variable in the successful group compared to the unsuccessful group (means and standard deviations in degrees were: successful, 268.20 and 6.35; unsuccessful, 284.40 and 13.32). Finally, body size differences were less in the successful group compared to the unsuccessful group (means and standard deviations in milligrams were: successful, 387.14 and 191.16; unsuccessful, 490.00 and 156.12).

Greater variation in the dorsal valve polar coordinate angle indicated that unstable valves and inconsistent spermatophore trajectories may have been associated with the unsuccessful HMCF group. But in the successful HMCF group, less variation in the dorsal valve angle indicated the valves were in a stable position, resulting in an optimal spermatophore trajectory as determined by the centrally located ventral valves. In the successful group, the close proximity of the ventral valve apex to the opening of the spermathecal duct suggested that a deep penetration increased the likelihood of successful transfer by shortening the distance traveled by the spermatophore tube (figure 5e). As mentioned under the cluster analysis, the ventral valves were aligned along the central axis of the bursa, so their proximity to the spermathecal opening probably overcame the dorsal deflection of the spermatophore tube dorsad as expected by Cohn and Cantrall (1974).

Body size differences in the HMCF cross can be explained by a mechanical model in which depth of penetration was proportional to the relative size of the male. In the unsuccessful group, larger body size

differences (Table 2) meant that males were small relative to females, penetrated less deeply, and failed to transfer spermatophore material. In the successful group, small body size differences were associated with larger males who penetrated deeply and transferred sperm successfully.

In the HMCF cross, body size differences also had an important effect in compensating for a relatively small aedeagus. The humphreysii aedeagus was much shorter than the cochisei bursa when compared to the humphreysii bursa [humphreysii aedeagus: 120-540 microns, humphreysii bursa: 165-585 microns, cochisei aedeagus: 205-720 microns, cochisei bursa: 270-750 microns (data from Cohn and Cantrall, 1974, and Bennack, unpublished).] The small humphreysii aedagus would not be expected to penetrate the elongate cochisei bursa as deeply as the cochisei aedeagus, but humphreysii males probably compensated for small aedeagal size using their larger relative body size to gain a mechanical or muscular advantage over the females. Whether the advantage was physical or a broadly defined "vigor" (e.g. Kence and Bryant, 1978; Van den Berg, 1986), the net result was a deep penetration that shortened the distance traveled by the spermatophore tube to a level similar to the intra-racial crosses (Tables 1 and 2). Given this critical data, it is not surprising the HMCF transfer frequency was so high. [Muscular or mechanical advantage used by males might have offset any effects of female choice suggested for the intra-racial crosses. If humphreysii males can subdue cochisei females, it would be difficult to distinguish mechanical explanations from those based on female choice.]

Once again, body size differences accounted for the apparently important effects of several genitalic variables. In the MANOVA (Table 4), the distance between the spermathecal duct opening and the dorsal

valve apex (i.e. the depth of dorsal valve penetration) was initially associated with successful spermatophore transfer. The ventral valve polar coordinate distance was also initially associated with transfer success. However, the effects of these variables were removed by size adjustment in the MANCOVA test (Table 4). The single genitalic variable relatively free of body size effects was again the dorsal valve polar coordinate angle ($p = 0.0001$; also see discriminant analysis).

In summary, the results of the discriminant and MANCOVA analyses suggest that despite the mismatched genitalia described by Cohn and Cantrall, the following factors may be responsible for the high frequency of spermatophore transfer in the HMCF cross:

(1) Similar male and female body sizes allowed the relatively small aedeagus to penetrate deeply into the bursa.

(2) Deep penetration shortened the distance traveled by the spermatophore tube such that intra-racial levels were observed (especially simialar to the CMCF level, Tables 1 and 2).

(3) Deep penetration probably offset the dorsal deflection of the spermatophore tube thought to be caused by the mismatched genitalia (Cohn and Cantrall, 1974).

The CMHF cross

In the CMHF cross, the average radial distance associated with the ventral valves ($p = 0.0015$), and the rotation of the ventral valves with respect to the bursa ($p = 0.0075$) were significant parts of the size-controlled discriminant model (Table 6). The average radial distance indicated the ventral valves were closer to the bursa in the successful group than in the unsuccessful group (Table 2 means and standard

deviations in microns were: successful, 22.62 and 9.92; unsuccessful, 26.78 and 11.00). The ventral valves were also more nearly parallel to the major axis of the bursa (relatively unrotated) in the successful group than in the unsuccessful group (means and standard deviations in degrees were: successful, 0.10 and 4.33; unsuccessful, 0.85 and 5.27). Body size differences were greater in the successful group than the unsuccessful group (means and standard deviations in milligrams were: inseminated, 675.00 and 233.03; unsuccessful, 667.50 and 179.38), but these size differences were not significantly associated with spermatophore transfer ($p = 0.1694$; MANOVA, Table 4).

In the successful group, ventral valves located close to the bursa may have made frequent physical contact with the bursal wall thereby stabilizing aedeagal movements. The outer surface of the valves was covered with minute spines which may have assisted in this function (Bennack, unpuplished data; a typical condition for many orthopteran species). In the unsuccessful group, valves located farther away from the bursa probably made less frequent contact. As discussed earlier, stabilization of the ventral valves probably reduced variability in spermatophore trajectories, and improved the likelihood of spermatophore transfer.

In the successful group, the ventral valve axes were also relatively unrotated with respect to the axes of the bursa (the major and minor axes of each reference system were approximately parallel). A lack of rotation indicated the genitalia were aligned for sperm transfer. In the unsuccessful group, deviations from parallel alignment (greater rotation) indicated that torsion or other more complex aedeagal movements had occurred during copulation. These genitalia were probably out of alignment and were unlikely to transfer spermatophore material

successfully.

Both the discriminant analysis and MANCOVA suggested the fit of the ventral valves was important in predicting low spermatophore transfer success in the CMHF cross. In the discriminant model, the significant estimates of ventral valve fit were the average radial distance, and the relative rotation of the valves. MANOVA and MANCOVA also verified these findings (Table 4). In addition, MANOVA and MANCOVA indicated the ventral valve polar coordinate distance was significantly associated with spermatophore transfer both before size adjustment (MANOVA, $p = 0.0012$) and after size adjustment (MANCOVA, $p = 0.0011$). The polar coordinate distance from the ventral valve center to the origin of the bursal reference system was greater in the unsuccessful group than the successful group (Table 2 means and standard deviations in microns were: successful, 2.41 and 2.16; unsuccessful, 4.96 and 3.09). These data suggested that if the ventral valves were not aligned along the center of the bursa, the spermatophore tube was not successfully guided toward the opening of the spermathecal duct.

Finally, in the CMHF cross MANOVA indicated no statistically significant body size effect on spermatophore transfer success ($p = 0.1694$), and MANCOVA suggested that certain ventral valve variables were independent of the effects of body size (rotation, $p = 0.0100$; polar coordinate distance, $p = 0.0011$; and average radial distance, $p = 0.0037$). Yet, male and female body sizes were strongly divergent in the CMHF cross (Tables 1 and 2), and the relatively small B. h. cochisei males often had considerable difficulty reaching around the abdomen and pentrating the bursa of B. h. humphreysii females (Appendix I). Such extreme body size differences probably resulted in a more shallow aedeagal penetration than was characteristic of the intra-racial crosses

(Tables 1 and 2, figure 5f), and greatly affected the stability of the aedeagus in the bursa when viewed in serial cross-sections. These data suggested the majority of copulating pairs were near the tolerance limit of large differences in male and female body size. Under such threshold conditions, adequate penetration for spermatophore transfer would have been about as likely as inadequate penetration. Consequently, the frequency of spermatophore transfer would have been about 50% (58% was observed) and no association between size differences and transfer success would have been expected (no effect was observed).

Despite the significance of the ventral valve fit in the discriminant and MANCOVA tests, it was still difficult to assign greater importance to the ventral valves in predicting spermatophore transfer when compared to the dorsal valves. Since the ventral valves were longer and appeared more frequently in the bursa than the shorter dorsal valves (22 of 114 sections had ventral valves; 69 of 114 sections had dorsal valves), the relative unimportance of the dorsal valves may have reflected statistical error associated with a small sample of dorsal valves. It was difficult to assert unequivocally that the dorsal valves did not influence spermatophore transfer, when the spermatophore tube had to pass through both sets of valves, and the dorsal valves were inconsistently present within the bursa (e.g. figures 12 and 13). Rather, it seemed more likely that:

(1) Instability of <u>both the dorsal and ventral valves</u> was associated with the success of spermatophore transfer in the CMHF cross,

(2) But the over-riding factor associated with the low transfer frequency in the CMHF cross was shallow aedeagal penetration

brought on by extreme differences in male and female body sizes.

The influence of male and female body size within the crosses.

Exploratory multiple regression analysis

Differences in male and female body size were clearly associated with spermatophore transfer in the crosses of B. humphreysii, and body size differences also explained many of the size-dependent estimates of genitalic fit. To better understand how body size differences might have contributed to a lock-and-key fit, an exploratory multiple regression technique was utilized. The relationship between size differences and spermatophore transfer was investigated as follows.

Within each cross, copulating pairs were ranked according to the difference in male and female body sizes, and grouped into three "size-difference" classes of approximately equal sample size. Spermatophore transfer frequencies were calculated for each group, and then assigned (as likelihoods of transfer) to copulating pairs based on their size-class identity. The transfer frequencies were deliberately biased to reveal body size effects. This technique increased the resolving power of the multiple regression with respect to body size differences, but at the expense of the other variables. Since the size-controlled effects of the other genitalic variables (notably the location of the aedegal valves) were already accounted for in the previous analyses, the use of size-biased data was justified to the extent that only body size effects were compared among crosses.

The following regression model treated spermatophore transfer frequency as a conceptually dependent variable (abbreviations are explained in Table 1):

(Size-biased spermatophore transfer frequency) = + b1 (dv to sp) + b2 (vv to sp) + b3 (unc bur) + b4 (dv axs) + b5 (vv axs) + b6 (dv bur avg) + b7 (dv bur var) + b8 (vv bur avg) + b9 (vv bur var) + b10 (dv pol dis) + b11 (dv pol ang) + b12 (vv pol dis) + b13 (vv pol ang) + b14 (dv rot) + b15 (vv rot)

where the b_is are regression coefficients associated with the independent genitalic variables.

The intra-racial crosses: HMHF and CMCF

In the intra-racial crosses of B. humphreysii, the regression of body size differences on spermatophore transfer frequencies provided: (1) useful information on the influence of size differences within races, and (2) a basis of comparison with the inter-racial crosses. [Since sexual dimorphism in body size was common within races, randomly chosen pairs of males and females in the intra-racial crosses were probably distributed about a size difference close to the average sexual dimorphism in the sampled populations.]

Regression on the size-biased data revealed a significant positive association between body size differences and spermatophore transfer frequency in the HMHF and CMCF crosses. With all genitalic variables in the regression, a unit increase in the difference between male and female size approximately doubled the likelihood of transfer in B. h. humphreysii (b = 1.82, p = 0.0001), and tripled the likelihood in B. h. cochisei b = 3.02, p = 0.0001). This relationship implied that smaller males and larger females were more likely to transfer spermatophore tubes than males and females that were closer in body size, given the bounds of intra-racial body size differences.

In the HMHF cross there was also a marginally insignificant negative relationship between the likelihood of transfer and unoccupied bursal area ($p = 0.0457$, $b = -0.80$). This negative relationship implied that as the bursa was left progressively unfilled by the aedeagus, spermatophore transfer correspondingly decreased. Although such a finding agreed with the lock-and-key hypothesis, the unoccupied area effect was not likely independent of body size differences and cannot be interpreted accurately. No other genitalic variables were associated with the frequency of spermatophore transfer in the size-biased regressions of either intra-racial cross.

The HMCF cross

In the HMCF cross, a significant negative association between body size differences and transfer frequency was observed. With all genitalic variables in the regression, a unit decrease in the difference between male and female body size increased the likelihood of spermatophore transfer one and one-half times ($b = -1.57$, $p = 0.0001$). This negative regression implied that as males became more similar in size to females, the likelihood of transfer correspondingly improved. No other genitalic variables were significantly associated with spermatophore transfer success in this size-biased regression.

The effect of body size differences in the HMCF cross was clearly in the opposite direction from the intra-racial crosses. In the HMCF cross, smaller differences in male and female sizes were associated with higher transfer frequencies, but in the intra-racial crosses, greater size differences were associated with higher transfer frequencies. As discussed under the discriminant analysis, females in the intra-racial crosses may have influenced spermatophore transfer at some stage of

copulation prior to ejaculation, refusing to engage in mutual courtship behaviors with males falling outside tolerance limits of body size (after Eberhard, 1985). Given the sexual size dimorphism occurring in natural populations of B. humphreysii, this explanation may not be unreasonable. In the inter-racial HMCF cross, males may have used muscular or mechanical advantage associated with large size to subdue mates, regardless of female choice. Since humphreysii males were larger on average than cochisei males (Table 1), the argument based on size-vigor may be appropriate. A deep and vigorous penetration probably compensated for the mismatched genital morphologies in the HMCF cross, allowing for a spermatophore transfer success comparable to the intra-racial crosses.

The CMHF cross

In the regression analysis of the CMHF cross, there was no significant association between body size differences and the frequency of spermatophore transfer ($p = 0.490$). Rather, transfer success was significantly associated with dorsal valve penetration ($b = 2.41$, $p = 0.001$), ventral valve penetration ($b = -3.26$, $p = 0.002$), the ventral valve polar coordinate distance ($b = 0.65$, $p = 0.014$), and the ventral valve medial axis length ($b = -0.59$, $p = 0.006$).

The lack of a body size effect indicated that variation in size differences had no statistical power in predicting the frequency of spermatophore transfer in the CMHF cross. This result seemed surprising, since transfer frequencies were clearly biased to reveal body size effects. Yet, recall that body size differences in this cross were more divergent than in the intra-racial crosses (Table 1), and that the

average transfer frequency (58%) was also considerably lower than in the other three crosses. Further, the effects of extreme body size differences were visible as a noticeably shallow penetration and an unprecedented absence of the dorsal valves from 60% of the bursal sections. These results suggested the CMHF cross represented a threshold level of body size differences, below which variation in size differences had little effect on spermatophore transfer. If most of the copulating pairs were near or below threshold conditions, this cross would have produced a uniformly low transfer frequency characterized by a statistically flat (non-significant) regression line. Although the threshold effect of body size differences seemed visually apparent in slides of the CMHF cross, it may have been statistically undetectable using a regression approach.

As in the discriminant and MANCOVA analyses, the multiple regression indicated that the ventral valves were more important than the dorsal valves in predicting spermatophore transfer success in the CMHF cross. But to conclude that the ventral valves were more significant than the dorsal valves has the same attendant problem as the other analyses. The non-significance of the dorsal valves may have reflected statistical error associated with a small sample size of these valves, rather than a biological lack of importance. Given the absence of dorsal valves in the bursa, the simplest conclusion was that extremely small males were unable to penetrate deeply with either set of valves, and that inadequate penetration of both sets of valves was important to low transfer success.

In summary, exploratory multiple regression analysis of the size-biased data revealed that body size differences may have exerted different mechanical effects among the four crosses:

(1) In the intra-racial crosses, an increase in female size relative to male size resulted in an increase in spermatophore transfer frequency. This relationship probably applied only within the tolerance limits of sexual size dimorphism and may have been related to female choice. (Specific data from female choice experiments would be required to demonstrate size-related tolerance limits.)

(2) In the HMCF cross, an increase in male size relative to female size resulted in an increase in spermatophore transfer success. This probably represented the ability of large males in the HMCF cross to gain mechanical or muscular advantage, compared to the relatively smaller males in the intra-racial crosses. Mechanical or muscular vigor may have permitted deep penetration, bringing the genital openings close together and compensating for the mismatched genitalia.

(3) In the CMHF cross, females were so large in relation to males that spermatophore transfer success was uniformly reduced. Probably for this reason, variation in size differences had no predictive association with the frequency of spermatophore transfer. The CMHF cross may have represented a lower threshold of size differences compared to the intra-racial crosses, such that penetration was shallow and spermatophore transfer correspondingly unlikley.

The lock-and-key hypothesis in Barytettix grasshoppers: a reappraisal.

In their monographic work on the genus Barytettix, Cohn and Cantrall (1974) noted that genitalic differences were insufficient to maintain complete mechanical isolation between B. h. humphreysii and B. h. cochisei. These investigators reported smoothly intergrading genitalic morphologies across several hybrid zones in southeastern Arizona and adjacent Mexico which they believed were the result of failed mechanical reproductive barriers. Electrophoretic data gathered by Bennack and Howard (unpublished) also confirmed the incomplete isolation of these taxa. Populations of B. h. humphreysii and B. h. cochiesi were differentiable by a fixed allele at only one of 18 allozyme loci sampled, but the frequency of the allelomorphs graded smoothly from 0% to 100% across inter-racial hybrid zones. Cohn and Cantrall were unable to determine why differences in genitalic morphology failed to establish complete mechanical isolation. Based on their thorough study of geographic variation in Barytettix, they concluded the genitalia in inter-racial crosses should be morphologically incompatible. Inter-racial hybridization contradicted the predictions of the lock-and-key hypothesis for these investigators.

My study of genitalic fit and the success of spermatophore transfer in B. humphreysii also corroborated incomplete isolation between the races, but suggested the mechanism of isolation is asymmetric. In this study, the CMHF cross showed a marked reduction in spermatophore transfer success compared to the intra-racial crosses, but the HMCF cross was at least as successful as the intra-racial crosses. According to my interpretation of the data analyses, mechanical factors such as the orientational stability of the dorsal valves partially explained

transfer success within each cross, but of even more importance were the differences in male and female body size that characterized the crosses. Body size differences explained several significant estimates of genitalic fit in the intra-racial crosses, and were probably responsible for the extremely shallow penetration of the aedeagus in the CMHF cross and the deep penetration in the HMCF cross.

Summary of genitalic fit in crosses of B. humphreysii

This study shows that the intra-racial crosses (HMHF and CMCF) were characterized by high levels of spermatophore transfer (84% in both cases). In addition, the genitalia of these crosses showed no major signs of incompatibility at the histological level. The curvature of the aedeagus corresponded well with the curvature of the bursal wall, short distances were observed between the aedeagal apices and the spermathecal tube opening (i.e. penetration of the dorsal and ventral valves was deep), and there was no unusual rotational instability of the valves. The ventral valves were aligned along the central axis of the bursa, which probably facilitated the delivery of the spermatophore tube. The ventral valves of the cochisei male were positioned to guide the spermatophore tube down the center of the bursa toward the anterior spermathecal opening, presumably following the deflection of the spermatophore by the medially-closed margins of the valves. Similarly, the ventral valves of the humphreysii male were probably positioned to deflect the spermatophore tube through the open region of the dorsal valves and toward the antero-dorsal opening of the spermathecal duct.

Male and female size differences, along with aspects of dorsal valve orientation, were the only variables associated with spermatophore transfer success in the intra-racial crosses. In the unsuccessful copulating pairs, the positional instability of the dorsal valves may have altered spermatophore trajectories from the optimal path suggested by Cohn and Cantrall (1974). Moreover, the dorsal valve orientation was the only measure of genitalic fit relatively free of the influence of body size.

Male and female size differences accounted for the effects of all other genitalic variables on spermatophore transfer, probably as a consequence of the closeness of the genital openings (i.e. the depth of aedeagal penetration). In addition, discriminant analysis and exploratory regressions suggested that males and females of more similar size were not as successful as males mated to relatively larger females. Although conjectural, this data suggested females may choose among copulating males based on body size differences. This hypothesis is briefly explored in chapter five.

In the inter-racial HMCF cross, the genitalia appeared to fit together well and transfer success (90%) was at least as high as the intra-racial crosses. The aedeagus penetrated deep into the bursa, which was slightly stretched as a consequence. This stretching added to the corresponding curvature of the genitalia. Deep penetration of the aedeagus was probably due to the relatively large size of the humphreysii male. Larger males may have had muscular or mechanical advantage over females in this cross that allowed deep penetration and successful spermatophore transfer, despite the short length of the aedeagus and mismatched genital morphologies. In particular, the proximity of the aedeagus to the anterior spermathecal opening decreased

the distance traveled by the spermatophore tube, and may have offset the consequences of the dorsally deflected spermatophore tube predicted by Cohn and Cantrall (1974). The only measure of genitalic fit unaffected by body size differences involved the positional stability of the dorsal valves. As in the intra-racial crosses, the instability of the dorsal valves may have interfered with the optimal trajectory of the spermatophore tube determined by the ventral valves.

In the inter-racial cross CMHF, the aedeagus only shallowly penetrated the bursa. Dorsal valve penetration was especially poor, but the orientation and fit of both sets of valves was dramatically affected. Shallow penetration was apparently a direct consequence of small male body size in this cross. Inadequate penetration may have increased the distance between the aedeagal and spermathecal openings, and resulted in a very low spermatophore transfer rate (58%) compared to the other crosses. However, within the CMHF cross, the lack of a statistically significant body size effect on the success of spermatophore transfer may have been due to a threshold effect. If many males in this cross were so small that adequate penetration (and successful spermatophore transfer) were about as likely as inadequate penetration (and failed transfer), then a statistically undetectable body size effect might have resulted.

Concluding remarks

The lock-and-key hypothesis may be too sophisticated to explain mating success in most organisms with intromittent modes of insemination because it places unrealistic expectations on the level of morphological complementarity that can be attained. In this study, quantitative variation in genitalic fit had an observable effect on spermatophore

transfer within each cross, but most of the genitalic variation associated with spermatophore transfer was explained at another level of morphological causation: the relative sizes of the copulating male and female. For example, in the intra-racial crosses HMHF and CMCF, differences in body size explained a number of genitalic variables that were associated with transfer success. More dramatically, in the HMCF cross, body size differences may have offset the effect of mismatched genitalia, allowing a deep penetration and a transfer rate as high as the intra-racial crosses. Conversely, in the CMHF cross, body size differences apparently compounded the effect of mismatched genitalia, producing a shallow penetration and a transfer rate much lower than the other three crosses.

It is my contention that differences in male and female body sizes were ultimately responsible for many of the effects of genitalic fit in the crosses of B. humphreysii, and were primarily responsible for the asymmetry in spermatophore transfer success between the inter-racial crosses. A lock-and-key explanation of mating incompatibility would have accurately predicted asymmetries in transfer success, had body size incompatibilities been treated as conventional genitalic characters. The deeper aedeagal penetration and adequate fit in the HMCF cross could have been anticipated as a consequence of relatively isomorphic body sizes, and the shallow penetration and poor fit in the CMHF cross would have been suggested by extreme differences in male and female body sizes.

If the morphology of genitalic fit depends on the relative differences in male and female body sizes, then investigators should treat body size differences as a character for consideration when studying mating incompatibility. Eberhard (1985) argued convincingly

that any unconventional character utilized in a mating context can respond to mating selection (e.g. secondary genitalia, antennae, rudimentray clasping wings, etc). In Barytettix grasshoppers, and perhaps many other organisms with intromittent modes of insemination, researchers may have failed to identify a most fundamental lock-and-key mechanism: the functional compatibility of male and female body sizes.

CHAPTER 5

AN EXTENSION OF THE KENCE-BRYANT-VAN DEN BERG MODEL OF MATING SUCCESS TO INTRA-RACIAL CROSSES OF B. HUMPHREYSII

This study of genitalic fit suggested that in the intra-racial crosses of B. humphreysii, larger females may have copulated more effectively when males were smaller, than when males and females were more similar in size. If differences in body size determined much of spermatophore transfer success, as this study indicated, then a simple knowledge of male and female body sizes, rather than a detailed knowledge of genital morphology, may be sufficient to predict spermatophore transfer.

Simple relationships between mating success and male and female characters have been observed in several insect groups. In particular, the Kence-Bryant-Van den Berg model has been used to explain assortative mating in houseflies (Musca domestica; Kence and Bryant, 1978), fruitflies (Drosophila melanogaster; Van den Berg, 1986), and soldier beetles (Chauliognathus pennsylvanicus; McCauley, 1981). According to this model, the tendency to mate varies among individuals such that the fitness of a mated pair is determined by differences in the mating "vigor" of each partner. Interestingly, body size is an important component of mating vigor in Musca and Chauliognathus.

The Kence-Bryant-Van den Berg model can also be extended to the intra-racial crosses of B. humphreysii. Specifically, if body size differences resulting from sexual size dimorphism affect the insertion of male aedeagi into female bursae as suggested by this study, spermatophore transfer in randomly mating populations should depend (minimally) on the distribution of body sizes within each sex. This

argument cannot be so readily extended to the inter-racial crosses because sexual dimorphism in body size is confounded with inter-racial differences in body size.

The initial formulation of the Kence-Bryant-Van den Berg model for the intra-racial crosses is

$$w = f\ (Z_f - Z_m) \qquad (1)$$

where Z_f is the size of the female, Z_m is the size of the male, and w is the likelihood of spermatophore transfer for the mated pair expressed as a function of the difference in female and male body sizes. To incorporate the distributions of within-sex body sizes into this expression, the equation can be restated as

$$w = \sum\sum p_i p_j\ (Z_{fj} - Z_{mi})^2 \qquad (2a)$$

where p_i and p_j are the proportions of a given body size occuring among males and females, respectively. Since $\sum p_i = 1$ and $\sum p_j = 1$, then $\sum\sum p_i p_i = 1$ so that

$$w = \bar{Z}_f^2 - 2\bar{Z}_f\bar{Z}_m + \bar{Z}_m^2 \qquad (2b)$$

Using the variance identity, $VAR = \bar{Z}^2 - \bar{Z}^2$, this model can be re-written as

$$w = VAR_f + VAR_m + (\bar{Z}_f - \bar{Z}_m)^2 \qquad (2c)$$

Since body size increases as a cubic function of length, and the likelihood of spermatophore transfer is conceptually linear, spermatophore transfer calculated from body size differences must be log-linearized and scaled to a probability range from 0 to 1. The final form of this model can be written as

$$w = 1/3 \log [VAR_f + VAR_m + (\bar{Z}_f - \bar{Z}_m)^2] - 1 \qquad (3)$$

Thus, fitness at the time of copulation can be broken down into additive components due to variation in female body size, variation in male body

size, and a dispersion measure of the difference in average within-sex body sizes. This model implies that in intra-racial populations mating randomly, spermatophore transfer success at or near the time of copulation can be predicted from the means and variances of male and female body sizes.

If body size differences are primarily responsible for spermatophore transfer success in the intra-racial crosses, then the distributional form of the Kence-Bryant-Van den Berg model should accurately estimate the transfer frequencies observed in the mating experiment. In addition, if smaller males and larger females copulate more effectively than males and females of similar size as this model of implies, then larger females and smaller males should be at a selective advantage in this study.

In this study, selection intensities reflected the strength and direction of phenotypic selection on male and female body sizes at the time of copulation. With respect to gender, selection intensity was the shift in mean body size expressed in standard deviation units (Arnold and Wade, 1984a, 1984b) between groups that were successful and unsuccessful in transferring spermatophore tubes. (The response to selection across generations was not measured.) In males, intensities of selection were -0.0644 in B. h. humphreysii, and -0.0030 in B. h. cochisei. In females, intensities of 0.0545 and 0.0799 were observed, respectively. Although small in magnitude, these estimates confirmed that shifts in mean body size due to differential spermatophore transfer were toward smaller males and larger females, a main premise of the Kence-Bryant-Van den Berg model as applied to Barytettix grasshoppers.

As suggested by the importance of body size differences in this study, the distributional version of the Kence-Bryant-Van den Berg model

accurately explained spermatophore transfer success in the intra-racial crosses of B. humphreysii. For B. h. humphreysii, a spermatophore transfer frequency of 0.8608 was calculated from the model. A value of 0.83857 was observed. In B. h. cochisei, a frequency of 0.8064 was calculated, and a value of 0.8438 was observed. These estimates were within error by 2.2 % and 3.7 %, respectively [error was calculated as (observed - expected)/expected X 100].

In conclusion, the application of the Kence-Bryant-Van den Berg model suggested that algebraic differences in male and female body sizes, if expressed in terms of the mean and variance, accurately accounted for spermatophore transfer in the intra-racial crosses of B. humphreysii. Such a simple predictive model provides a powerful tool for studying natural populations of Barytettix grasshoppers, and adds strength to the main thesis of this study: that differences in male and female body size were the primary determinants of spermatophore transfer in B. humphreysii, and should be regarded as critical components of a lock-and-key mechanism.

CHAPTER 6

FUTURE DIRECTIONS FOR *BARYTETTIX* RESEARCH

Sexual selection and eco-geographic speciation: an overview.

In a series of important papers, Lande (1980, 1981, 1982) has shown that sexual selection can produce rapid divergence in characters associated with sexual isolation and speciation. In Lande's models, runaway sexual selection depends on the existence of genetic covariation between female mating preferences and male secondary sexual traits. A balance between sexual selection and natural selection characterizes populations at equilibrium, yet the nature of the balance permits many combinations of female choice and male traits to evolve (Arnold, 1985). When multiple male characters are being selected, the possible evolutionary outcomes of sexual selection are enormously varied.

According to Fisher (1958), traits under sexual selection may evolve rapidly in a self-reinforcing process of runaway selection. Lande's models also confirm this qualitative prediction. In particular, if the genetic covariation between mating preference and the selected trait is sufficiently large compared to the additive genetic variation in the trait, then sexual selection can shift a character away from its optimal value under natural selection at geometric or exponential rates (Engen and Saether, 1985; Lande, 1981). Instability and the subsequent triggering of the runaway process are favored by a combination of large genetic covariation between mating preference and the sexually selected trait, weak natural selection on the sexually selected trait, and strong stereotypic preferences in the sex that chooses mates (Arnold, 1985).

Runaway sexual selection is expected when the sexually selected trait is sex-limited, and also when its homologue is present in the opposite sex. Thus, mating preferences and sexual dimorphism can evolve concurrently in natural populations (Lande and Arnold, 1985). Under sexual selection, the average of the male and female homologous character should evolve rapidly, but the evolution of sexual dimorphism should be slowed by genetic correlations between the sexes (Lande, 1980; Arnold, 1985).

The operation of sexual selection also implies the existence of tolerance limits on female mating preferences and sexually selected male traits (Lande, 1981). In this manner, sexual selection can promote the genetic cohesion of a breeding population by constraining the amount of variation that exists within a population at any one time. Since speciation converts within-population variation into between-population variation (Darwin 1878; Lewontin, 1974; Wade 1977), widespread sexual selection has the potential to accelerate the accumulation of variation among populations and contribute to speciation. Traditionally, the evolution of inter-racial variation has been considered a by-product of the allopatric speciation process (Muller, 1940; Mayr, 1963) or a direct consequence of selection against hybridization in zones of contact (Gulick, 1888, Sturtevant, 1938; Dobzhansky, 1940, 1970).

Lande's work shows how sexual selection can interact with ecological processes to promote population divergence and rapid speciation. Especially susceptible to this process are secondary sexual characters used in premating isolation. According to Arnold (1985), sexual selection:

(1) Permits multiple evolutionary outcomes for female mate preference and male sexual traits. This increases the

likelihood that different populations will respond to slightly different mating cues.

(2) Allows transient differences in sexual traits to persist among populations during the slow final aproach to equilibrium. This prolongs the time over which selection can act on population differences.

(3) Enables sampling drift among populations to shift preferences and selected characters to new stable states. A similar exploration of ecologically adaptive states is predicted by Wright's (1931) shifting balance model.

(4) Exaggerates geographic variation associated with clines, patchy environments, and ecological boundaries where natural selection is known to vary spatially (Endler, 1977, 1986).

Sexual selection, eco-geographic speciation and B. humphreysii.

The interaction of sexual selection and eco-geographic processes of speciation may be especially important in the evolution of Barytettix grasshoppers. In this study, intra-racial crosses of B. humphreysii displayed a potential basis for mate choice that depended on genitalic incompatibility and sexual dimorphism in body size. Although B. humphreysii races differ eco-geographically in body size and genitalic morphology, they produce hybrids in sympatry that intergrade smoothly across ecotonal contact zones (Cohn and Cantrall, 1974). These data, coupled with the observation that selection favors larger females and smaller males within races, suggested that B. humphreysii may be diverging along ecological gradients by the interaction of sexual selection and spatially varying natural selection. Because these grasshoppers readily hybridize and are easily reared in the lab (Bennack

and Cohn, unpublished data), critical estimates of the genetic basis of character divergence can be obtained through conventional quantitative genetic methods. Thus, Barytettix humphreysii offers an excellent opportunity to study the interaction of sexual selection and incipient eco-geographic speciation.

Selection on body size and genitalic characters: general predictions.

The investigation of genitalic fit in Barytettix reported in this study suggests body size and genitalic morphology should respond to mating selection both within and between races. In intra-racial populations, mating selection should favor females that are larger than males, thus maintaining a functionally efficient size dimorphism during copulation. In addition, genitalia should be selected that ensure an adequate fit between the male aedeagus and the female bursa. As a consequence of these joint constraints, male and female genitalia properly scaled with body size should be selected as a correlated unit. Mate choice should also be a significant component of spermatophore transfer success. If active mate choice is present, selection should favor individuals who assess mate quality on the basis of body size and/or the tactile recognition of genitalic fit (Eberhard, 1985). However, if mate choice is passive, then selection should minimally be a function of the distribution of male and female phenotypes in the population as predicted by the Kence-Bryant-Van den Berg model of mating success (Kence and Bryant, 1978; Van den Berg, 1986).

When races meet in sympatry, mating selection should also act on body size and genitalic characters. If mate choice is active, then mates should choose members of their own race because intra-racial matings are functionally efficient with respect to body size. Inter-

racial matings, which fall outside the normal range of expected body sizes, should be discriminated against. If mate choice is not limited by within-race expectations of body size, or if mate choice is passive, then studies of genitalic fit and spermatophore transfer suggest that selection may be asymmetric. In particular, selection should disproportionally favor B. h. humphreysii males because they can mate successfully with females of either race. Barytettix h. cochisei males should be selected against in contact zones because they mate relatively inefficiently with B. h. humphreysii females. (The evolutionary response to selection is not being considered here.)

Selection may also favor the tactile recognition of genitalic fit in contact zones. Since ventral aedeagal valve morphology diverges between the races (figure 4), mates may favor the fit of ventral valves in intra-racial matings, but discriminate against this coupling in inter-racial matings. In hybrids having intermediate morphologies, selection on the fit of the genitalia may be less severe.

Selection on body size and genitalic characters: testable hypotheses.

The evolutionary response of genitalic and body size characters to selection is a complex process. Minimally, it depends on the heritable basis of variation in the morphological traits and the accumulated effects of natural selection over ontogeny. In addition, eco-geographic patterns of character covariation may be affected by selection during hybridization (Lofsvold, 1986).

The task of characterizing these components of an evolutionary response to selection in Barytettix can be focused on a set of specific predictions. Although the following predictions are not exhaustive, they do provide a framework for detailed studies of the interaction of

sexual selection and speciation in Barytettix.

Genetic Parameters:

(1) Patterns of ontogenetic character covariation should differ between races (Lofsvold, 1986).

(2) Body size and genitalic morphology within sexes should be substantially heritable and genetically correlated.

(3) Genetic correlations between the sexes should exist for body size, and also between aedeagal and bursal dimensions.

Selection in Allopatry:

(1) Phenotypic selection at the time of copulation should favor smaller males and larger females (contrast with McCauley, 1981).

(2) Phenotypic selection at the time of copulation should favor aedeagi and bursae of similar dimensions.

(3) Genitalic and body size characters should be selected as a correlated unit.

(4) Over ontogeny, natural selection should enhance the development of sexual dimorphism in body size.

Interactions in Zones of Sympatry:

(1) The genetic covariance structure should be disrupted (be more variable) in zones of sympatry compared to allopatry. (Lofsvold, 1986).

(2) Phenotypic selection at the time of copulation should favor larger males and smaller females.

(3) Phenotypic selection at the time of copulation should favor the recognition of ventral aedeagal valve morphology.

The theoretical literature is rich with accounts of how, and under what conditions, reproductive characters and sexually dimorphic traits should evolve as a consequence of mating success. Barytettix is a particularly exciting test case for models of sexual selection, sexual dimorphism, correlational selection, and clinal speciation because much is already known about reproductive morphology and spermatophore transfer. A thorough understanding of the genetic basis of these quantitative traits is still required. Only then can the evolutionary response to selection at the time of mating be studied in depth. Because genitalic and body size characters can be readily measured in allopatry and sympatry, critical estimates of these genetic parameters can be made.

Evolutionary models make very specific predictions about the consequences of selection on sexual characters. These range from the rapid divergence of genitalia (Eberhard, 1985), to the joint evolution of sexual dimorphism and mate choice (Lande and Arnold, 1985), to the promotion of clinal speciation as sexual and natural selection interact (Lande, 1982). Barytettix grasshoppers offer a promising opportunity to study these evolutionary processes in natural populations.

APPENDIX I.

BEHAVIORAL OBSERVATIONS OF COPULATING *BARYTETTIX* GRASSHOPPERS

During the experiments conducted at the Southwest Research Station of the American Museum of Natural History, the following effects of body size on copulation were observable among the crosses of *Barytettix humphreysii*.

(1) In the intra-racial crosses (HMHF and CMCF), the general tendency was for males to initiate an immediate mounting attempt on the females, usually within the first five minutes after being placed in the mating cages. Males required relatively few attempts (approximately one to three) to penetrate the female bursa before success was achieved. The female was seldom observed (or seemed inclined) to dislodge the mounted male with kicks from the hind femora. There were a few occasions when females dislodged intra-specific males, but often a repeated mounting attempt by the male resulted in penetration for the duration of the 90 minute observation required by this study. Dislodged males appeared to have some difficulty in reaching around the abdomen of the female to make contact with the female genitalia and penetrate the bursa. There was some indication this difficulty was related to the relatively small body size of the male compared to the female.

(2) In the inter-racial crosses, body size differences between copulating males and females noticeably affected the males ability to mount and penetrate the female bursa. In the HMCF cross, the male was much larger with respect to the female than in the intra-racial crosses. Thus, *humphreysii* males were

easily able to reach around the abdomen of the cochisei female and penetrate the bursa. Females were never observed to dislodge males in the HMCF cross. although there were occassions where females appeared to be kicking at the males with the hind femora. In the CMHF cross, males were much smaller than there respective female mates. These small males were frequently dislodged after the initial mounting attempt, often before penetration was achieved. Females occasionally refused to open the ovipositor valves allowing access to the bursa during the initial phases of mounting. The small body size of the male noticeably affected the ability to reach around the female abdomen and penetrate the bursa. This was especially obvious during the mating trials when many males never achieved penetration (and thus were not used in this study). The effects of small male size were also apparent at the time of specimen fixation when genitalia thought to be in copula actually fell apart due to a lack of penetration.

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