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VARIATION IN GENETIC ARCHITECTURE OF CALLING SONG AMONG POPULATIONS OF *ALLONEMOBIUS SOCIUS, A. FASCIATUS*, AND A HYBRID POPULATION: DRIFT OR SELECTION?

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Abstract.—Predictions using quantitative genetic models generally assume that the variance-covariance matrices remain constant over time. This assumption is based on the supposition that selection is generally weak and hence variation lost through selection can be replaced by new mutations. Whether this is generally true can only be ascertained from empirical studies. Ideally for such a study we should be able to make a prediction concerning the relative strength of selection versus genetic drift. If the latter force is prevalent then the variance-covariances matrices should be proportional to each other. Previous studies have indicated that females in the two sibling cricket species *Allonemobius socius* and *A. fasciatus* do not discriminate between males of the two species by their calling song. Therefore, differences between the calling song of the two males most likely result from drift rather than sexual selection. We test this hypothesis by comparing the genetic architecture of calling song of three populations of *A. fasciatus* with two populations of *A. socius*. We found no differences among populations within species, but significant differences in the **G** (genetic) and **P** (phenotypic) matrices between species, with the matrices being proportional as predicted under the hypothesis of genetic drift. Because of the proportional change in the (co)variances no differences between species are evident in the heritabilities or genetic correlations. Comparison of the two species with a hybrid population from a zone of overlap showed highly significant nonproportional variation in genetic architecture. This variation is consistent with a general mixture of two separate genomes or selection. Qualitative conclusions reached using the phenotypic matrices are the same as those reached using the genetic matrices supporting the hypothesis that the former may be used as surrogate measures of the latter.

Key words.—Genetic correlation, genetic covariance, genetic variance, heritability, quantitative genetics.

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Calling song in insects is frequently an important component of mate recognition and mate attraction (Walker 1957; Alexander 1975; Otte 1977). Within Orthoptera various components of calling song have been shown to be important in attracting females (Forrest 1983; Hedrick 1986; Weidmann and Keuper 1987; Cade and Cade 1992; Hedrick and Dill 1993; Tuckerman et al. 1993). Thus within a species or population we might expect calling song to be under relatively strong selection. However, variation between species might represent genetic drift (Walker 1974) or selection for characteristic differences if the two species overlap in their range and their calling songs are so similar that females cannot readily distinguish between them (Howard 1993).

Calling-song components typically show continuous variation, and genetic analyses suggest that this variation is polygenic (Butlin and Hewitt 1986; Hedrick 1988; Webb and Roff 1992; Mousseau and Howard 1997). For such traits the methods of quantitative genetics are an appropriate means of analysis. Under selection the calling-song components will change in a predictable fashion provided it can be assumed that the genetic architecture remains constant. For there to be no change in the **G** matrix as a result of selection requires that selection be very weak. Genetic drift can cause a change in the **G** matrix, but this will be roughly a proportional change (Lande 1979; Lofsvold 1988). This latter condition ensures that the genetic correlations (r_G) remain constant (because each is the ratio of a genetic covariance and the square root of two genetic variances), but does not ensure that the heritabilities (h^2) remain constant, because these are the ratios of the additive genetic variances to the phenotypic variances. Thus for the assumption of constancy for both r_G and h^2 to hold we require that both the **G** matrix and the phenotypic variances remain constant or all change by the same factor. If there is no change in the environmental variance, the phenotypic variance will change as a result in the change in the genetic variance, but this change will not be proportional to the change in **G**. We have little theory to guide us as to when to expect significant variation in genetic architecture (Turelli 1988; Arnold 1992; Roff 1997). At present we need empirical studies of variation in genetic architecture at several taxonomic levels (for a review of studies to date see Roff 1997, pp. 110–116). Because it is potentially under strong selection within populations and species, but possibly not between species, calling song is a trait that deserves particular attention with respect to the stability of genetic architecture.

The two cricket species *Allonemobius fasciatus* and *A. socius* are closely related, inhabit the same type of habitat, and have the same phenology, but are geographically separated over much of their ranges (Howard 1983). However, in several places they come together along a mosaic hybrid zone in which hybrids are formed less often than expected under random mating (Howard 1986; Howard and Waring 1991). There are highly significant quantitative differences between the species in the components of the calling song (Howard and Furth 1986; Mousseau and Howard 1997). However, studies of female phonotaxis suggest that conspecific sperm

precedence is the cause of present-day isolation rather than variation in male calling song (Howard and Gregory 1993; Gregory and Howard 1994; Doherty and Howard 1996). Thus, a priori, we hypothesise that genetic differences between the species in calling song are the result of genetic drift rather than sexual selection.

The purpose of the present study was to determine whether there is significant variation in the genetic or phenotypic variances and covariances among populations of the two species, between the two species, and between either species and a hybrid population. On the basis of the phenotypic data outlined above, we predict that the matrices between species will either not differ or will be proportional. Predictions for the comparisons involving the hybrid population are more difficult and less certain, because although we can expect an increase in genetic variation we have no theory to tell us how this variation will be expressed. However, it does seem likely that the conjunction of different sets of genomes will produce variance-covariance matrices that differ from each other and are unlikely to differ across all components by a simple constant of proportionality.

Methods

A detailed description of the collection sites, rearing protocol, and song recording methods are presented in Mousseau and Howard (1998) and here we present only an overview. Crickets were collected from six populations within a 50-km radius of Camden, New Jersey. In this region, *A. fasciatus* and *A. socius* meet and form a mosaic hybrid zone. Species compositions were determined using the allozyme assay described in Howard and Waring (1991). Two populations (M23 and M26) were composed of *A. socius* genotypes; three were composed of *A. fasciatus* genotypes (NS, RS, and LF); and a sixth population (HF) contained a mix of *A. socius, A. fasciatus*, and hybrid genotypes.

Between 100 and 400 late-instar nymphs were collected from each site during August 1990 and returned to the laboratory for the completion of development in a common-garden environment of 29°C, 11:13 hr L:D. Following the attainment of sexual maturity, males and females were randomly paired (within populations) to form P_1 parentals. Hatchlings were caged in 10 × 15 × 20 cm plastic boxes containing a water vial, a slice of carrot, a small amount of crushed Purina Cat Chow (original formula), and strips of unbleached paper towels at a density of fewer than 30 nymphs per cage. Between 50 and 200 F_1 full-sib families per population were successfully reared. Each family was reared until final eclosion in a common cage, and at eclosion individuals were transferred to individual cages where they were housed until they began calling. Although this procedure cannot eliminate the possibility of common environmental effects on the calling song from rearing conditions during the nymphal stage, it ensured that there was no effect of common environment during the adult phase.

A 30-sec segment of the calling song of all male parents (P_1) and four or five randomly chosen F_1 male offspring were recorded at between 25.5°C and 27°C. Males were recorded while sequestered individually in a plastic cage (17.5 × 12 × 6 cm) covered with fine mesh netting. Most males were recorded at one to two weeks of age post adult eclosion. Details of the method of recording and song analysis are given in Mousseau and Howard (1998). The calling song of *Allonemobius* males is made up of a series of chirps, each consisting of a number of pulses (see fig. 1 in Olvido and Mousseau 1995). From a 10–20 sec portion of each recording the following seven calling song parameters were estimated: mean number of pulses per chirp (MP), mean chirp period (CP), mean interchirp interval (ICI), mean carrier frequency (= dominant frequency, FREQ), mean pulse period (PP), mean pulse rate (PR), and mean pulse duration (PD). To avoid the problem of redundancy due to the part-whole correlation between CP and ICI, the latter was dropped from the present analysis. Only sires in which all components were recorded were used; the total sample size was 504 sires and 1961 sons.

Both carrier frequency and temporal parameters of the calling song have been shown to be important in attracting females in orthoptera (see references in Benedix and Howard 1991; Crnokrak and Roff 1998), but the relative importance of the individual song components measured in the present analysis have not been examined in *Allonemobius*.

Statistical Analysis

Our interest is in additive genetic variation that will be manifest in the field; therefore, we used son-on-sire regression as a screening tool, retaining for further analysis only those traits in which there was at least a significant correlation between son and sire. The estimation of additive genetic (co)variance from son-on-sire regression assumes that the genetic correlation between laboratory and field is one (see below) and is therefore only a lower bound on the estimate. Further, the power of single-parent regressions are very low (Roff 1997). Because of these two weaknesses, we compared the matrices using the full-sib data (i.e., sons). Additive genetic variance estimates from full sibs may be inflated by maternal or dominance variance; therefore, results must be considered cautiously. However, significant variation among populations or species does indicate some source of variation, even if it remains to be confirmed that it is due to additive genetic effects alone.

Both carrier frequency and temporal parameters of the calling song have been shown to be important in attracting females in orthoptera (see references in Benedix and Howard 1991; Crnokrak and Roff 1998), but the relative importance of the individual song components measured in the present analysis have not been examined in *Allonemobius*. The elimination of traits that had low levels of additive genetic variance was made to increase the power of the statistical comparisons among the matrices. Of course, traits under strong selection are likely to be those that show little genetic variance. However, including them in the analysis will make the matrices more similar and thus increase the probability of not rejecting the null hypothesis that the genetic architecture of the songs has not changed (either by drift or selection).

Son-on-Sire Regressions

The additive genetic variance obtained under field conditions, V_{AF}, is related to those in the laboratory, V_{AL} (or any

two environments) according to the relationship (Riska et al. 1989)

$$V_{AF} = \frac{4b^2 V_{PF}^2}{r_A^2 V_{AL}}, \quad (1)$$

where b is the slope of the son-on-sire regression, V_{PF} is the phenotypic variance in the field, and r_A is the additive genetic correlation between the two environments. If b is zero, there can be no additive genetic variance in the field. A statistically significant slope indicates that there is additive genetic variance in the field, although the precise amount depends on the other components in the equation.

The potential existence of variation among populations or species can be assessed by including "population" or "species" (*fasciatus, socius*, or hybrid) as dummy variables in the regression:

$$X_{Son} = a + bX_{Sire} + cD + dX_{Sire}D, \quad (2)$$

where X is the trait, D is a dummy variable designating population or species, and a–d are fitted constants. A significant additive effect indicates only a phenotypic difference between the groups, whereas a significant interaction term (d) is evidence for differences in genetic effects because it indicates significant differences in the slopes of the species- or population-specific regressions. However, because the field populations may have experienced different conditions during development, a significant interaction term can be considered only suggestive of variation among the groups in additive genetic variance.

The above relationships can be extended to consider the genetic correlation between two traits by regressing trait Y of the sons on trait X of the sires. A significant correlation between X and Y is putative evidence for a genetic covariance between the two traits. Similarly, a significant interaction between populations or species suggests the presence of variation in the additive genetic covariance.

Full-Sib Analysis

There are three matrices to be compared: (1) the matrix containing the heritabilities and genetic correlations, which we shall refer to as the **H** matrix; (2) the matrix containing the genetic variances and covariances (the **G** matrix); and (3) the matrix containing the phenotypic variance and covariances (the **P** matrix). For each of these, the following two statistical analyses were performed.

Analysis 1.—In this analysis the null hypothesis is that the elements in one matrix from one population or species (**H**, **G**, or **P**) do not differ from the corresponding elements in the corresponding matrix of another population or species. This hypothesis can be extended to multiple populations, H_0: $\theta_{ij} = \theta_{ik}$, $j \neq k$, where θ_{ij} is the ith element in the jth or kth population or species. The above suggests the following test statistic for two populations:

$$T = \sum_{i=1}^{C} |\hat{\theta}_{i1} - \hat{\theta}_{i2}| \quad (3)$$

where $\hat{\theta}_{ij}$ is the estimate of θ_{ij} and C is the number of elements in the matrix (sum of the number of diagonal elements plus the number above or below the diagonal: $C = 0.5n[n + 1]$, where n is the number of traits). The above statistic can be extended to three or populations by computing all pairwise comparisons. To estimate the probability, P_T, of obtaining a value of T at least as large as that observed, T_{obs}, we used randomization. Families were assigned randomly to each population, T computed for the randomized dataset, T_r, and compared to T_{obs}. For each test 4999 randomizations were generated, and P_T was estimated as

$$P_T = \frac{1 + \text{number of randomizations in which } T_r > T_{obs}}{5000}. \quad (4)$$

As a check on the robustness of the test, we also used the squared deviations; the results did not differ and we present only the results using the absolute deviations. We also calculated the probabilities associated with each element of the matrix (designated as P_E). Although, because of the multiple estimations these values cannot be used individually, they do provide an indication of whether differences between the matrices arise because of a few strikingly variable elements or because of overall differences (the situation is analogous to the examination of individual cell values in a χ^2 test).

Because the scale of measurement for the traits is not constant (cf. mean number of pulses per chirp vs. mean carrier frequency), we standardized the phenotypic variances of the traits by dividing each by the phenotypic variance of the entire dataset, which retained the variation among families, populations, and species but standardized the contribution to T. To prevent variation between population or species arising from the randomization as a result of differences in mean values, before comparison, the trait values within a population were standardized to zero by subtraction of the trait mean for the particular population (Roff 1997, p. 102).

Analysis 2.—To test for proportionality, we consider the model in which the elements in one matrix from one population or species (**H**, **G**, or **P**) are linearly related to the corresponding elements in the corresponding matrix of another population or species,

$$\theta_{i1} = A + B\theta_{i2}. \quad (5)$$

TABLE 1. Analysis of son on sire using either population or "species" (*Allonemobius fasciatus, A. socius,* hybrid) as a factor. Table shows the probabilities associated with the regression components and the overall R^2. Where the interaction term was not significant (ns), the results for the additive model are shown.

Trait (X)	X	Factor	Interaction	R^2
		Factor = population		
MP	0.0002	0.0441	0.0307	0.56
CP	0.0001	0.0000[1]	0.0222	0.57
FREQ	0.0000[1]	0.0000[1]	ns	0.32
PP	0.0055	0.0000[1]	ns	0.13
PD	0.9794	0.002	ns	0.03
PR	0.6421	0.0166	ns	0.02
		Factor = "species"		
MP	0.0000[1]	0.0082	0.0096	0.56
CP	0.0000[1]	0.0000[1]	0.0023	0.56
FREQ	0.0000[1]	0.0000[1]	ns	0.56
PP	0.0013	0.0000[1]	ns	0.11
PD	0.7371	0.1135	ns	0.01
PR	0.0055	0.0000[1]	ns	0.13

[1] $P < 0.00005$.

TABLE 2. Regression analysis of trait Y of son on trait X of sire using "species" as a factor. Table shows the probabilities associated with the regression components and the overall R^2. Where the interaction term was not significant (ns), the results for the additive model are shown.

Sire's trait	Son's trait	Sire effect	Species effect	Interaction	R^2
CP	MP	0.0082	0.0000[1]	0.0108	0.55
MP	CP	0.0001	0.0000[1]	0.0001	0.55
MP	FREQ	0.5225	0.0000[1]	ns	0.29
FREQ	MP	0.5231	0.0207	0.0067	0.54
CP	FREQ	0.9851	0.0000[1]	ns	0.29
FREQ	CP	0.0067	0.0007	0.0026	0.54

[1] $P < 0.00005$.

The above formulation suggests a linear regression approach. However, the specification of the dependent and independent variables is arbitrary, and simple linear regression is excluded because, in general, there will be approximately the same variance in the both the dependent and independent variables. The solution to this is to use reduced major axis (RMA) regression. Tests on the significance of A or B were made using the same randomization procedure as described above (probabilities designated P_A and P_B, for tests on A and B, respectively). Under the randomization procedure $A = 0$ and $B = 1$, and, therefore, deviations of A were assessed by comparing $|A_{obs}|$ with $|A_r|$, whereas deviations of B were assessed by comparing $|B_{obs}-1|$ with $|B_r - 1|$. A nonsignificant A but significant B indicates that the matrices are proportional and that the proportionality constant is significantly different from 1. If both A and B are significant the elements of the two matrices are linearly related but not proportional. The above test is not readily extended to more than two groups, but this was not necessary in the present analysis.

TABLE 3. Probabilities (P_E) obtained from randomization tests of the equality of the elements of the variance-covariance matrices among the three *Allonemobius fasciatus* populations and between the two *A. socius* populations. Note that these data are used to examine the pattern of variation and not to test for statistical significance of individual elements.

Trait(s)[1]	H	G	P
	A. fasciatus (LF, NS, RS)		
MP	0.04	0.04	0.93
FREQ	0.18	0.26	0.69
CP	0.42	0.50	0.53
MP, FREQ	0.73	0.73	0.90
MP, CP	0.26	0.39	0.47
CP, FREQ	0.92	0.61	0.42
	A. socius (M23, M26)		
MP	0.11	0.14	0.76
FREQ	0.59	0.62	0.90
CP	0.08	0.10	0.77
MP, FREQ	0.82	0.91	0.73
MP, CP	0.01	0.10	0.17
CP, FREQ	0.22	0.21	0.81

[1] Entries for individual traits show results for heritability (**H**) or variance (**G, P**). Entries for paired traits show results for genetic correlations (**H**) or covariances (**G, P**).

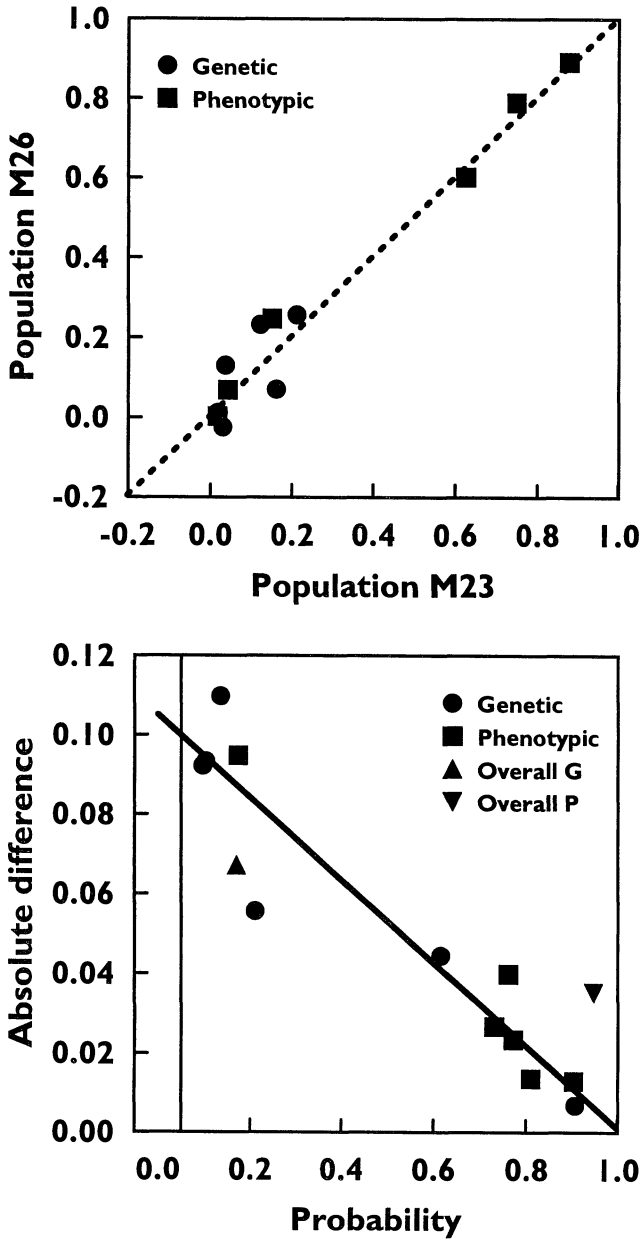

FIG. 1. (Top) Plot of the genetic and phenotypic variances and covariances of the two *Allonemobius socius* populations (M26, M23). (Bottom) Plot of the element by element absolute differences against the probability value obtained from the randomization. Also shown for comparison are the values of T and P_T ("overall" on graph legend). The vertical line is drawn at $P_E = 0.05$, and the sloping line is the regression line.

The Distribution of Hybrids in the Parents and Offspring

Although random mating of individuals from the pure populations may reasonably mimic the mating combinations found in the wild population, randomly mating individuals from the hybrid population might be biased toward higher levels of hybridization than are actually found in the field. The difference so caused can be assessed by comparing the hybrid index score (computed as the sum of the species-specific alleles at four allozymes, where alleles specific to *A.*

TABLE 4. Heritabilities and genetic and phenotypic correlations for the combined populations of *Allonemobius socius* and *A. fasciatus* and the single hybrid population. Sample sizes are ("species," number of families, number of individuals): *socius,* 174, 704; *fasciatus,* 212, 759; hybrid, 118, 498.

		Heritability, genetic and phenotypic correlation (SE)[1]		
Trait 1	Trait 2	*A. fasciatus*	*A. socius*	Hybrid
MP	MP h^2	0.35 (0.08)	0.46 (0.08)	0.80 (0.10)
CP	CP h^2	0.42 (0.09)	0.37 (0.08)	0.98 (0.09)
FREQ	FREQ h^2	0.73 (0.09)	0.53 (0.08)	1.06 (0.10)
MP	CP r_G	0.30 (0.19)	0.60 (0.14)	0.82 (0.05)
	r_P	0.23 (0.04)	0.29 (0.04)	0.53 (0.05)
MP	FREQ r_G	0.13 (0.14)	0.07 (0.15)	−0.53 (0.11)
	r_P	0.09 (0.04)	0.07 (0.04)	−0.21 (0.06)
CP	FREQ r_G	−0.09 (0.11)	0.01 (0.13)	−0.53 (0.09)
	r_P	0.02 (0.04)	0.01 (0.04)	−0.28 (0.06)

[1] Estimates and standard errors computed using the jackknife (Roff and Preziosi 1994; Simons and Roff 1994).

fasciatus were assigned a score of +1 and alleles specific to *A. fasciatus* were assigned a score of −1; Howard and Waring 1991) of the parents with that produced under the given mating regime. The hybrid index of the parents was measured and that for the offspring generated by taking the mean of the two parents. It was not our intention to do a detailed statistical analysis of the difference, but simply to see if visually there was an obvious difference between the distribution of scores in parents and offspring. If large differences are apparent, then the results pertaining to comparisons using the hybrid data must be interpreted with caution, particularly when extrapolating the results to the wild.

RESULTS

Son-on-Sire Regressions

The son-on-sire regressions were carried out using first "population" as a factor (i.e., LF, RS, NS, M23, M26, HF) and second using "species" by combining the *fasciatus* populations and the *socius* populations (i.e., three "species": *socius, fasciatus*, and hybrid). Where the interaction term was nonsignificant the regression was recalculated using only the additive model (Table 1). Pulse duration (PD) showed no significant son-on-sire regression, whereas pulse repetition rate (PR) was significant when the data were grouped by species, but not when grouped by population. Pulse period (PP) of sons was significantly correlated to that of their father for both analyses, but accounted for only a very small proportion of the variance (11–13%) and showed no significant interaction effects (Table 1). The three remaining song components (CP, MP, FREQ) all showed significant correlations, and in two cases (MP, CP) there were significant interaction terms. These results were not Bonferonni corrected because the traits are themselves correlated (Manly 1997). The rationale for the present analysis is to select traits for the matrix analysis and hence the absolute level of significance is not critical. What is important is the amount of variation accounted for by a trait. The three traits CP, MP, and FREQ all account for relatively large amounts of variance (> 30% in all cases, and > 50% in all but one case; Table 1). Although there is little difference between the analysis using population as the factor versus species as the factor, the latter analysis does give somewhat better results in generally accounting for more variation (Table 1). On the basis of the significant regressions and the amount of variation accounted for, we selected CP, MP, and FREQ for further analysis (although PR showed one significant correlation, it accounted for such a small fraction of the variance that to include it in further analysis would likely seriously reduce the power of the tests).

The across-trait regressions were carried out only with the data grouped by species. The significant sire component and interaction effect suggests the presence of additive genetic covariance between CP and MP and variation among species (Table 2). Results for the other two covariances (CP vs. FREQ, MP vs. FREQ) are unclear: when FREQ is the independent (sire) variable there is indication of species differences (significant interaction terms), but the interaction terms are not significant when FREQ is the dependent (son) variable (Table 2).

Comparison of Matrices

There was no significant variation among the three *fasciatus* populations for any of the matrices (P_T = 0.28, 0.36, 0.71 for **H**, **G**, **P**, respectively). None of the individual elements showed any indication of marked variation, with the overall probabilities arising from a general similarity of the three populations (Table 3). For the two *socius* populations there was no significant variation in the **G** or **P** matrices (P_T = 0.17, 0.95, respectively), but a slight indication of a difference between the **H** matrices (P_T = 0.04). This difference appears to arise from the genetic correlation between CP and MP (P_E = 0.01, Table 3). However, there was no significant variation in either *A* (P_A = 0.40) or *B* (P_B = 0.17) for the **H** matrix. The marginal significance of **H** (without Bonferroni correction) and the lack of significant variation in the reduced major axis regression argues for, at best, only minor differences between the two populations.

There is no analytical method of estimating statistical power in the present case, but the probabilities associated with the individual elements can be used to estimate the absolute difference required to produce a significant difference under the given sample sizes. For *A. socius* there is a clear correlation between the populations in both the genetic and phenotypic (co)variances (Fig. 1). There is also a highly significant linear relationship between P_E and the corresponding absolute difference (absolute difference = 0.1051 − 0.1041 P_E, r = 0.89, $P < 0.00001$: covariance analysis indicated no significant effect [$P > 0.1$] of the type of variance [phenotypic or genetic]). Using this relationship the absolute value at which P = 0.05 is estimated to be 0.10. The average genetic (co)variance is 0.10, which indicates that the present test can distinguish an absolute difference that is of the same size as the genetic (co)variance. Thus, quite substantial differences would have to exist between the populations before they were statistically distinguishable. However, the observed mean absolute difference is 0.0386, which is 37% of the of the average genetic (co)variance. Therefore, the observed absolute difference is considerably below that required to produce significance, as evidenced also by the high overall probability value (P_T = 0.17; see also Table 3). The mean phenotypic

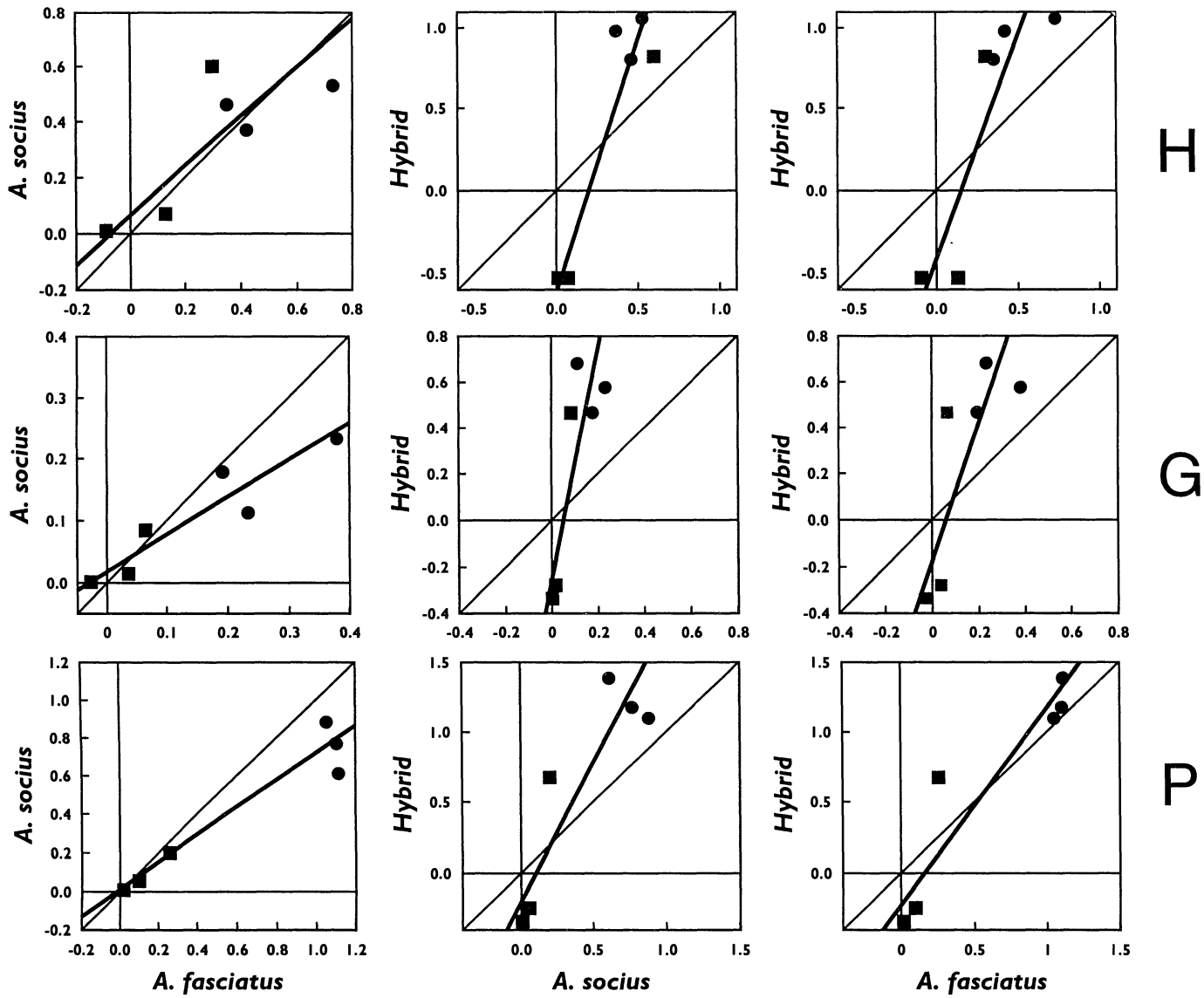

FIG. 2. Pairwise plots of the elements of the **H** (top row), **G** (middle row) and **P** (bottom row) matrices. ● h^2 or variances, ■ r_G or correlations. The thick line in each plot is the reduced major axis regression line.

(co)variance is 0.38, and thus the present test can determine a significant differences as small as 26% of the average phenotypic (co)variance. The mean absolute difference is 0.0308, which is only 8% of the average phenotypic (co)variance.

The above approach does not extend readily to three populations and so in the case of *A. fasciatus* we analyzed two populations (NS and RS), which represent the two smallest sample sizes (150 individuals measured from NS, 218 individuals from RS, and 391 individuals from LF). Because the number of individuals measured in the NS and RS populations is substantially less than the number of *A. socius* individuals measured (339 in M23, 365 in M26), we would expect a reduction in power. As before, there was a highly significant linear relationship between absolute difference and P_E (absolute difference = $0.2401 - 0.2573P_E$, $r = 0.91$, $P < 0.0001$: (co)variance type had no significant effect). From the forgoing equation the absolute difference detectable at the 5% level is 0.23. The mean genetic (co)variance is 0.16, and thus a difference less than 143% of the average value will not be declared statistically significant. The observed mean absolute difference is 0.0873, which is 55% of the average genetic (co)variance. Although the observed difference is large, it is considerably below the value required for significance. This analysis is conservative in that it ignores the contribution of the third, largest population.

Because of the lack of significant variation among the populations within species, we combined the populations, after correcting each trait within a population to a mean of zero. The heritabilities and genetic correlations of the two species are very similar and markedly different from the hybrid population, which shows considerably larger amounts of genetic variation (Table 4). Pairwise plots of the heritabilities and genetic correlations (Fig. 2) suggests that *A. socius* and *A. fasciatus* do not differ, whereas *A. fasciatus* and *A. socius*

TABLE 5. Randomization tests of the hypotheses of equality and proportionality between the various pairwise combinations of matrices.

Components	Equality (P)	Null hypothesis: Intercept = 0 Estimate	Intercept = 0 P_A	Slope = 1 Estimate	Slope = 1 P_B
		A. fasciatus, A. socius			
H	0.4450	0.06	0.5748	0.89	0.6500
G	0.1802	0.02	0.4128	0.60	0.0072
P	0.0002	0.01	0.6832	0.71	0.0002
		A. socius, hybrid			
H	0.0002	−0.62	0.0002	3.08	0.0002
G	0.0002	−0.25	0.0002	4.96	0.0002
P	0.0002	−0.20	0.0002	1.97	0.0002
		A. fasciatus, hybrid			
H	0.0002	−0.42	0.1382	2.78	0.0028
G	0.0004	−0.18	0.1544	2.94	0.0078
P	0.0002	−0.22	0.0054	1.39	0.0056

differ from the hybrid population at least by a constant of proportionality.

The **H** matrix of *A. fasciatus* did not differ significantly from that of *A. socius* (P_T = 0.45), and there was no evidence of a difference from the analysis of the RMA regression (Table 5, Fig. 2). In contrast, while there was no significant variation in the **G** matrix when tested for equality (P_T = 0.18), there was evidence for proportionality of the matrices from the RMA regression analysis (Table 5, Fig. 2). This variation is very evident in the **P** matrix for which the probability of obtaining two matrices from the same statistical population as deviant as observed is less than 0.0002. The RMA regression analysis indicates that hypothesis that the matrices are proportional cannot be rejected (Table 5, Fig. 2). Variation between the two species appears to arise primarily from differences in the variances rather than the covariances (Fig. 2, Table 6).

As suggested by visual inspection of the heritabilities and genetic correlations (Table 4, Fig. 2), the **H** matrices of both *A. fasciatus* and *A. socius* differ significantly from that of the hybrid population (P_T = 0.0002 for both comparisons). These differences arise from substantial variation in all elements of the matrix (Table 6). The hypothesis of proportionality cannot be rejected for the comparison between *A. fasciatus* and the hybrid population because the intercept of the RMA regression is not significantly different from zero but the slope differs significantly from one (Table 5). In contrast, both the intercept and slope of the RMA regression differed significantly for *A. socius* versus the hybrid population (Table 5, P_A = 0.0002, P_B = 0.0002).

Both the **G** matrices and the **P** matrices of the two pure species differed significantly from the hybrid population, with the **G** matrix following the same pattern as found for the **H** matrix (i.e., proportionality between *A. fasciatus* and the hybrid population, but significant differences in both slope and intercept between *A. socius* and the hybrid population; Table 5). For the **P** matrix there were significant differences in slope and intercept of the RMA regression between either species and the hybrid population (Table 5). Unlike the comparison between *A. socius* and *A. fasciatus*, the differences between the two species and the hybrid population arise from both variation in the variances and the covariances (Table 5).

TABLE 6. Probabilities (P_E) obtained from randomization tests of the equality of the elements of the variance-covariance matrices among the *Allonemobius fasciatus, A. socius,* and the hybrid population. Note that these data are used to examine the pattern of variation and not to test for statistical significance of individual elements.

Trait(s)	**H**	**G**	**P**
	A. fasciatus, A. socius		
MP	0.3378	0.8036	0.0002
FREQ	0.1142	0.0595	0.0814
CP	0.6974	0.0708	0.0002
MP, FREQ	0.7486	0.6524	0.4672
MP, CP	0.2562	0.6932	0.3236
CP, FREQ	0.5734	0.4758	0.8528
	A. socius, hybrid		
MP	0.0206	0.0006	0.0020
FREQ	0.0002	0.0004	0.0352
CP	0.0002	0.0002	0.0002
MP, FREQ	0.0008	0.0003	0.0003
MP, CP	0.0358	0.0003	0.0003
CP, FREQ	0.0004	0.0003	0.0004
	A. fasciatus, hybrid		
MP	0.0016	0.0038	0.5938
FREQ	0.0212	0.0842	0.7366
CP	0.0002	0.0002	0.0730
MP, FREQ	0.0004	0.0002	0.0002
MP, CP	0.0016	0.0002	0.0002
CP, FREQ	0.0004	0.0002	0.0002

Comparing the Hybrid Parents and Offspring

From the hybrid population 167 males and 167 females, which were collected from the field, were assayed for their hybrid index. There was no difference between the sexes (P = 0.47, Mann-Whitney U-test; P = 0.86, Kolmogorov-Smirnov test), and so these were combined. The distribution of

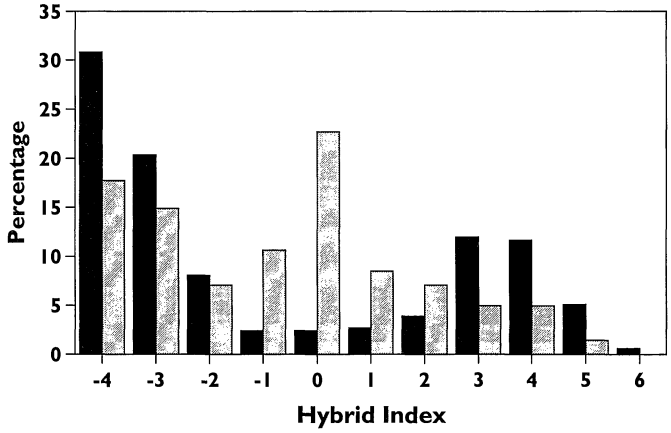

FIG. 3. Distribution of the hybrid index of adults from the hybrid population (Black bars, n = 334) and the mean offspring hybrid indexes predicted using the sire-dam pairs (gray bars, n = 141; this number differs from that reported in Table 4 because not all pairs were used in the present analysis). A score of −4 is a "pure" *Allonemobius socius*, whereas a score of +6 is a "pure" *A. fasciatus*.

hybrid index scores is bimodal, with the largest mode at the *A. socius* end of the spectrum (Fig. 3). The distribution computed from the means of the pairs used in the genetic analysis differs markedly from the parental distribution, having a large mode at a hybrid score of zero (Fig. 3). Thus, as suggested in the Methods, the distribution of offspring used in the present study were biased toward an excess of hybrids. This means that the high degree of genetic variation observed in the laboratory cross overestimates that which is likely to be found in the field hybrid population. Nevertheless, the distribution of hybrid scores among the field adults does show that gene flow between the two species does occur.

Discussion

The hypothesis that genetic variation between *A. socius* and *A. fasciatus* in calling-song components results from genetic drift cannot be rejected. Overall, there are no significant differences between the heritabilities and genetic correlations. The latter is to be expected from the proportionality of the genetic variances and covariances. However, algebraic equality of the heritabilities also requires that the phenotypic variances increase proportionally. Because the phenotypic variance equals additive genetic variance plus environmental variance, the phenotypic variance will change in concert with the additive genetic variance and at least a rough proportionality will be maintained. Whereas there is no clear separation between the genetic correlations and heritabilities, the genetic variances are significantly larger and deviate more between species than the genetic covariances (Fig. 1). It is indeed the genetic variances that result in the proportional difference rather than the covariances.

As would be expected, the heritabilities and genetic correlations of the hybrid population are considerably larger in magnitude (absolute value) than those of either *A. socius* or *A. fasciatus* (Table 4). Also as predicted, the variance-covariance matrices of the hybrid population differ very significantly from the two pure species. Most importantly the differences are not proportional, which indicates either selection acting on the hybrid population or a general mixture of two genomes that themselves differ due to genetic drift. The considerable increase of genetic variation in the hybrid population does suggest the potential for rapid evolutionary change in song structure within the hybrid zone. Doherty and Howard (1996) suggest that there is no selection on the females to avoid heterospecific males because females mate repeatedly and hence are likely to mate with a conspecific at some point and because the female gains a nuptial feeding from the male either by feeding on the tibial spurs or the spermatophore.

The random mating of individuals from the hybrid population appears to have resulted in higher levels of hybridization than actually found in the field. Therefore, the differences between the hybrid population and the two pure species are probably overestimated. However, the field-collected indexes do demonstrate that the hybrid population is composed of hybrid individuals and thus that the present results are not simply an artifact of mixing two separate species.

Genetic analyses are very labor intensive and time consuming. Because the genetic correlation is a component of the phenotypic correlation, Cheverud (1988) suggested that at least in some instances the latter could be used in place of the former. If true, this would considerably reduce the difficulties of analysis. For morphological traits, the phenotypic correlations do appear to be suitable surrogate measures of the genetic correlations (Roff 1997, pp. 96–100). In this context the question is "can the **P** matrix be used as a surrogate measure of the **G** matrix?" In all but one case the same answer is obtained using the **P** matrix as that obtained using the **G** matrix (Table 5). The one exception is that the hypothesis of equality cannot be rejected for the comparison of the **G** matrix between *A. socius* and *A. fasciatus*, whereas it is rejected using the **P** matrix (Table 5). However, the RMA regression analysis using **G**, which indicates a proportionality significantly different from one, supports the conclusion of a significant deviation from equality arrived at using the **P** matrix. Thus, because of its greater statistical power, the **P** matrix, although possibly biased (the slopes of the GMA regressions tend to be underestimated), may actually be a better indicator of genetic variation than the **G** matrix. Certainly the **P** matrix can be used to give a preliminary indication of variation. If the analysis indicates a very low probability of difference between the population/species/taxa being compared, it is unlikely that anything but a massive genetical analysis will pick up a difference.

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