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FIELD MEASUREMENTS OF NATURAL AND SEXUAL SELECTION IN THE FUNGUS BEETLE, *BOLITOTHERUS CORNUTUS*

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Abstract.—Selection on three phenotypic traits was estimated in a natural population of a fungus beetle, *Bolitotherus cornutus.* Lifetime fitness of a group of males in this population was estimated, and partitioned into five components: lifespan, attendance at the mating area, number of females courted, number of copulations attempted, and number of females inseminated. Three phenotypic characters were measured—elytral length, horn length, and weight; there were strong positive correlations among the three characters. Selection was estimated by regressing each component of fitness on the phenotypic traits.

Of the three traits, only horn length was under significant direct selection. This selection was for longer horns and was due mainly to differences in lifespan and access to females. The positive selection on horn length combined with the positive correlations between horn length and the other two characters resulted in positive total selection on all three characters.

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An important goal of current evolutionary biology is to study selection occurring in natural populations. Many studies have demonstrated selection in nature, but very few of these studies have measured lifetime fitness, separated direct selection acting on a character from indirect selection on correlated characters, or identified selective agents (Endler, 1986). The first shortcoming can only be overcome by long-term field studies; in animals, lifetime reproductive success of individuals has just begun to be estimated (Clutton-Brock et al., 1982; Fincke, 1982; Banks and Thompson, 1985; Hafernik and Garrison, 1986; Koenig and Albano, 1987). Two recent theoretical advances make the second and third problems easier to resolve.

First, the methods of Lande and Arnold (1983) allow one to estimate selection acting on several correlated traits simultaneously, separating direct from indirect selection. Direct selection for an increase in character value (i.e., magnitude of a quantitative character) results from a positive relationship between that character and fitness, when other correlated characters are held constant. A character under direct selection can be called the "target" of selection (Price, 1984; Price et al., 1984). Indirect selection can be explained as follows. If there is direct selection for an increase in the value of character x, then an individual with a high value of x will tend to have high fitness. If character y is positively correlated with character x, then that same individual will have a high value of y, thus creating indirect selection for an increase in y. The total selection acting on a character is the sum of direct and indirect selection.

Second, identifying selective agents can be facilitated by dividing lifetime fitness into biologically meaningful components (e.g., lifespan, access to females, insemination success) that can be more easily linked to the agents responsible for fitness differences (e.g., predation, male competition, female choice) (Arnold and Wade, 1984*a*; Koenig and Albano, 1986). Thus, by estimating as many components of fitness as possible over the lifetime of an organism and relating variation in these components of fitness to several different characters simultaneously, the specific agents that cause direct selection on individual target characters can often be identified.

In the study reported here, I estimated direct and total selection on three phenotypic traits during the adult lifetime of male *Bolitotherus cornutus. B. cornutus* is a tenebrionid beetle that lives throughout its life cycle on polypore shelf fungi (principally *Ganoderma applanatum*). Eggs are laid singly on the surface of the fungi, and larvae feed by tunneling through the interior (Liles, 1956). After pupation (about three months

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after oviposition), adults emerge and are active at night on the surface of the fungi, spending the daylight hours behind bark or in holes in the fungi. Eggs are laid from late May through August. Adults may emerge late in the summer from eggs laid in the spring, but most larvae overwinter and emerge as adults the next year (Liles, 1956). Adults can live for several years (Pace, 1967).

Individual beetles do not come out on the surface of the fungi (where virtually all matings occur) every night; thus only a subset of males are active on any given evening. The remainder spend the night behind the bark or in holes in the fungi. Males apparently come out on the surface of the fungi solely for mating, since they cannot feed on the upper surface of the fungi and do nothing on the surface other than mating and aggressive behavior (pers. observ.). Male *B. cornutus* possess pronotal horns, which vary allometrically with body size (Brown and Siegfried, 1983). The horns are used in aggressive encounters between males. Females lack horns and show little aggressive behavior.

Courtship begins when a male climbs onto the back of a female. Females appear to have little control over which males court them. A male's courtship frequency is determined at least in part by male competition, since males often aggressively chase each other away from fungi containing females (pers. observ.). After ten minutes to several hours of courtship, the male attempts to mate. The female has very little control over this stage, since she is rarely able to prevent a courting male from attempting to copulate. The number of attempts a male makes is determined by at least two factors: male competition (since males disrupt the courtships of other males before the courting male can attempt to mate) and, possibly, male choice (since some courtships are abandoned by males without an attempt [pers. observ.; Pace, 1967; Brown et al., 1985]).

While females probably have little control over which males attempt to copulate with them, they have complete control over which males successfully mate and transfer a spermatophore. Females have a heavy plate at the abdominal tip that acts as a trap door; it must be opened before a male can mate (Conner, 1987). Thus, female cooperation is necessary for mating to occur, because males cannot force entry. After a successful mating, a male guards the female for two to five hours; this distinct behavior is a reliable indicator of successful mating and spermatophore transfer (Conner, 1987).

A large natural population of *B. cornutus* near Ithaca, NY, was studied for two mating seasons (1984 and 1985). This species proved to be an excellent subject for studies of selection, since all of the biologically relevant components of adult male mating success were measurable (see below). Since growth does not occur during the adult stage of *B. cornutus,* there were no complications due to correlations between the characters measured and age. One potential problem was that generations overlap in this species. Cross-sectional (short-term) studies on populations with overlapping generations that are increasing or decreasing in size can lead to erroneous estimates of the magnitude and even the direction of selection (Travis and Heinrich, 1986). In the present study, however, fitness was measured over all or most of the adult lifespan, and the population size was stable throughout the study (see below).

Materials and Methods

Field Observations

The study population of *Bolitotherus cornutus* was located on a dead tree with 15 living fungi (*Ganoderma applanatum*). Beetles were collected when they were first seen and taken to the laboratory for marking and measuring; every adult beetle in the population was marked. The lengths of the horns and elytra (wing covers) of all males in this population were measured (to ± 0.2 mm) with an ocular grid on a stereo microscope. All beetles were also weighed (to ± 0.2 mg) with a Mettler® balance and given an individual paint mark on the elytra (with Testors® white enamel). This treatment did not seem to disturb the beetles, and 90% of marked beetles were observed at least once after release.

The population was first marked in late July 1983. To estimate lifespans, the beetles were censused from one to three times per night on a total of 190 nights. Censuses were performed on 35 nights from 22 July to 21 September 1983, 77 nights between 19 May

and 28 August 1984, 59 nights between 16 May and 27 August 1985, and 19 nights between 27 May and 28 July 1986.

To estimate mating success, the population was observed for approximately 1,000 hours on 136 nights throughout the 1984 and 1985 mating seasons (the same nights on which censuses were conducted in those two years; see above). Since the summer of 1985 was colder than 1984 (mean nightly low temperature 10.9°C vs. 12.3°C, $t = 2.1$, $P < 0.025$) and beetle activity was sharply reduced at temperatures below 10°C (pers. observ.), the beetles were observed on fewer nights in 1985. Observations were conducted on about 90% of the nights when weather conditions were suitable for beetle activity. Most observations were made during the time when the beetles were most active, between midnight and 7:00 A.M. A headlamp, equipped with an infrared filter (Kodak Wratten #89B) that transmits some far-red visible light, was used for illumination. The beetles remained active all night under illumination from this light, in contrast to the complete disruption of beetle behavior that was caused by a less filtered red light. All courtship, copulation, and male fighting behavior was recorded. Matings did not occur behind the bark or in holes in the fungi (where the beetles that were not on the fungal surface were), because there was not enough room for mating to take place in these areas (this was verified by observations using a fiber-optics viewer).

Selection was estimated using the 67 males that first appeared on the tree between 25 September 1983 and 6 June 1985. Thus, the study animals made up a "cohort" of beetles that emerged after most mating activity was over in 1983 and before the onset of most mating in 1985. The beetles from late 1983 and early 1985 were included because a beetle that emerges as an adult in the late fall and overwinters is not biologically different from one that overwinters as a last-instar larva and emerges in the spring; they hatch from eggs that were laid at about the same time, and their reproductive lives start in the spring. Indeed, a beetle that is first seen in the spring may have emerged as an adult in the fall and stayed inside the fungus until spring. Six beetles from 1983 and 13 from 1985 were included in the cohort.

A total of 134 males were present during the 1984–1985 observation period; thus, the study cohort represents half of the beetles present. The other 67 males were first observed either before 25 September 1983 or after 6 June 1985. The direct-selection analyses were performed separately on the cohort and on all 134 males; both sets of analyses are presented here. The population size was stable from 1984 to 1985; of the 134 total males, 94 were present in 1984 and 93 in 1985, with 53 males present in both seasons.

In 1985, migration between trees in the study population was measured by locating all other trees ($N = 17$) supporting *G. applanatum* in the approximately 5-hectare patch of woods containing the main study tree. These 17 trees were approximately 200–400 meters from the main study tree. All beetles on these trees were measured and marked as above; a total of 75 males were marked at the 17 trees. These trees were censused every night that observations were conducted during the first two-thirds of the 1985 mating season. During the last third of the 1985 season, the four trees closest to the main study tree (200–250 m away) were censused three times per night, while the other trees were censused twice weekly.

Components of Fitness in Male B. cornutus

Lifetime male mating success in *B. cornutus* was partitioned into five components. The first, adult lifespan (W_1), was estimated as the number of nights between the first and last sightings of a beetle, not including nights on which observations were not conducted. Since the beetles were observed on about 90% of the nights when they were active, this method of estimating lifespan reflects the relative amount of time available to the beetles for reproduction.

Three different estimates of lifespan were made. The first estimate was lifespan during the 1984 and 1985 seasons only (the symbol for this estimate is W_1). This was not the best estimate of individual lifespans that could be made, however, because 17 of the 67 males (25%) were still alive at the beginning of 1986, after the detailed observations made in the 1984 and 1985 seasons had ended. The measured lifespans of these

males were truncated, because the observations ended before the males died. Therefore, the second lifespan estimate (W_1') was made using the additional census data from 1986. In the partitioning of fitness into components, W_1 was used instead of W_1', since the fitness components other than lifespan were not measured in 1986. Of the 17 males that were alive in 1986, eight (12%) were still alive at the end of July 1986, when the population censuses were terminated. For this reason, the third lifespan estimate was made. The true mean lifespan of the entire cohort was estimated using the LIFETEST procedure (SAS Institute, 1982), which took into account the fact that the lifespans for eight of the males were truncated. LIFETEST cannot provide lifespan estimates for individuals, so this estimate was used only to see how well the other two estimates represented the true mean lifespan of the cohort.

The second fitness component is attendance at the mating area (W_2), which was estimated as the number of nights a beetle was on the fungi divided by its estimated lifespan, i.e., the percentage of nights during a male's life that it spent in the mating area. Since there was no apparent reason for the males to be out on the fungi other than mating, this is a component of sexual selection and is the first component of male access to females.

The third fitness component is the number of females courted (W_3). This is the second component of male access to females, and it was estimated by dividing the number of females courted by attendance, which equalled the average number of females courted per night for each male.

The fourth fitness component is the number of copulations attempted by a male (W_4). This is the third component of male access to females, and it was estimated as the number of copulations attempted divided by the number of females courted; in other words, this component is the proportion of a male's courtships that end in a mating attempt.

The fifth and final fitness component is the number of females a male inseminates (W_5), which was estimated as the proportion of male mating attempts that result in transfer of a spermatophore. Since the success of a mating attempt is controlled completely by the female, this component of fitness may be determined by female choice. It is possible, however, that males can sense when a female is receptive (Conner, 1987); thus, this component of fitness could also be determined partly by male competition for access to receptive females.

Notice that each component is a prerequisite for the next; a male must be alive before he can attend the mating area, he must be in attendance before he can court a female, he must court before he can attempt to copulate, and he must make an attempt if he is to inseminate a female successfully. Also note that these components naturally separate the biologically relevant components of lifetime mating success, e.g., longevity (W_1), attendance at the mating area (W_2), access to mates due to male competition (W_3, W_4, and [possibly] W_5), and female choice of mates (W_5). As required by Arnold and Wade's (1984*a*) model, the product of these components equals total fitness (Table 1). The denominator of each component is the numerator of the component before it; thus, when the components are multiplied, all the elements cancel except number of females inseminated, which I have defined as total fitness.

Clearly, this omits the last component of adult male fitness, which is the number of eggs that are fathered per female inseminated. This component of fitness could not be directly estimated; however, related studies of *B. cornutus* suggest that the number of females inseminated is probably a good estimate of the number of eggs fathered (Conner, 1987). Female *B. cornutus* were inseminated about every nine days on average in this population, whereas the mean interval between ovipositions in the laboratory was about three days. Thus, females tend to lay at least one egg per insemination. The last male to inseminate a female usually fathers most of the subsequent offspring (Conner, 1987). Therefore, an insemination is very likely to lead to offspring, and the number of females inseminated should be related to the number of eggs fathered. There are several sources of variability in how many eggs a male sires per insemination, however; the possible effects of this variability on the estimates of selection are discussed below.

Measures of Selection

The methods employed in this study estimate selection on phenotypes without considering inheritance; that is, they separate selection occurring within a generation from the evolutionary or genetic response to selection across generations. Also, only directional selection was estimated in this study.

Selection Gradients.—A selection gradient (β) measures the relationship between fitness and an individual character, holding other correlated characters constant (Lande and Arnold, 1983). Selection gradients therefore estimate the direct selection on each character after the effects of indirect selection on all correlated characters have been removed (assuming that all correlated characters have been measured). Selection gradients presented in this paper are the standardized partial regression coefficients for each character, which were generated in a multiple regression of relative fitness on the three phenotypic characters measured (Lande and Arnold, 1983). (Relative fitness is an individual's fitness divided by the population mean fitness.) Each of the phenotypic character values was first standardized so that the mean equalled 0 and the variance equalled 1 (z scores; Sokal and Rohlf, 1981). For example, a selection gradient of 0.5 for character x means that, on average, an increase of one standard deviation in character x was associated with an increase in relative fitness of 50%. Since selection gradients are partial regression coefficients, this analysis provided significance levels for the gradients and the total variance in fitness that is accounted for by all the characters combined (the r^2 value for the regression).

Selection gradients for each component were generated with a separate multiple regression. Males with zero fitness for a given component were not included in the regressions for subsequent components, since these males were "eliminated" by selection acting in the previous component. For example, males that did not court females ($W_3 = 0$) could not attempt to copulate; thus to include these males in the fourth component would not separate selection occurring in the third and fourth components. Five of the males had zero fitness in the third component (i.e., they courted no females), and nine had zero fitness in the fourth component (i.e., they did not attempt to copulate). Selection gradients for total fitness were calculated by regressing relative total fitness (number of females inseminated) against the three phenotypic characters for all 67 males. Since the gradients for each component were calculated with separate multiple regressions and the gradients for the last two components do not include all 67 males, the component gradients do not necessarily sum to the total selection gradient (see Arnold and Wade [1984*a*], Kalisz [1986], and Koenig and Albano [1987] for discussions of this issue).

One problem with this approach is that, if the correlations between the x variables are large, it is difficult to demonstrate the significance of any of the selection gradients. In the formula for the standard error of a partial regression coefficient (the selection gradient here), the squared correlations among the x variables are subtracted from the denominator (C. McCulloch, pers. comm.; Snedecor and Cochran, 1967). If these correlations are large, the standard error becomes large, and it is harder to show that the coefficient is different from zero. Therefore, there is a high probability of type II error (i.e., of not detecting selection when it does exist). The high standard errors also mean that the actual values of the selection gradients should be interpreted with caution. In addition, it becomes more difficult to show significance of the entire regression model with increasing numbers of correlated x variables. This is because each additional x variable increases the degrees of freedom, without increasing the explained sums of squares in proportion to the increase in degrees of freedom (since the added x variable is correlated with the x variables already included in the model).

Selection Differentials.—Selection differentials estimate the shift in the population mean value of a quantitative character due to total selection, both direct and indirect. Total selection is the sum of the direct selection on a character and the direct selection on all other correlated characters, weighted by the phenotypic correlation be-

TABLE 1. Mean male fitness values and standard deviations for each fitness component. W_1 is the mean lifespan for the main study period only (1984–1985), and W_1' is the mean lifespan including the census data from 1986; both of these estimates include individuals whose lifespans were truncated. W_1'' is the estimated total population mean lifespan, taking the eight truncated data points from W_1' into account (see text for details). W_{2-5} is total sexual selection (the product of components 2–5). The five multiplicative fitness components are in boldface; the product of these equals total lifetime inseminations.

Symbol	Fitness component	Mean	SD
W_1	**Lifespan** (days)	**56.2**	34.5
W_1'	Lifespan including 1986 (days)	65.5	46.1
W_1''	Estimated total lifespan (days)	69.0	53.2
W_2	**Attendance/lifespan**	**0.65**	0.19
W_3	**Courtships/attendance**	**1.01**	0.47
W_4	**Copulation attempts/courtships**	**0.60**	0.25
W_5	**Inseminations/copulation attempts**	**0.24**	0.22
W_{2-5}	Inseminations/lifespan	0.10	0.08
	Total lifetime inseminations	5.31	6.20

tween each character and the character of interest. In the case of three correlated characters,

$$i_1 = \beta_1 + \beta_2 r_{12} + \beta_3 r_{13} \qquad (1)$$

where i_1 is the standardized selection differential on character 1, β_1 is the standardized selection gradient for character 1, and r_{12} is the phenotypic correlation between characters 1 and 2. $\beta_2 r_{12}$ represents the indirect selection on character 1 that is due to direct selection on character 2. Note that Equation (1) represents the standard relationship between simple and multiple regression coefficients.

The selection differentials given in this paper are the regression coefficients from a simple regression of relative fitness on the standard score of each phenotypic character. The selection differential is defined as the covariance between relative fitness and the character (Lande and Arnold, 1983). The regression coefficient equals the covariance divided by the variance of the character; with standard scores, the variance is 1, so the coefficient equals the differential. Note that these differentials are equivalent to the standardized univariate selection gradient and the selection intensity, as defined by Falconer (1981). The differentials for each fitness component were calculated by a separate regression and thus are similar, but not exactly equal, to the partitioned selection intensities of Arnold and Wade (1984*a*). The differentials used here have the advantage of being simply related to the gradients (β) by Equation (1) but, like these gradients, may not sum to the differential for total fitness. Another advantage of these differentials is that they can be assigned standard errors and be tested for significance like any regression coefficient. Since these are simple regressions, the standard errors are not inflated by the covariances between the x variables as in multiple regressions (see above), so that small selection differentials are more likely to be significant than small selection gradients.

All calculations, regression analyses, and statistical tests were performed with the Minitab (Ryan et al., 1982) and SAS (SAS Institute, 1985) statistical packages.

RESULTS

Migration

Eight males were observed to make ten migrations among trees during the 1985 season (two males moved twice). Eight of these (80%) were short-distance moves of less than 25 m. Four of the 18 total trees with fungi in the woodlot (22%) contained only dead fungi. Six of the ten emigrations were from these four trees; thus, an average of 1.5 males (6/4) were observed leaving the trees with dead fungi in 1985, while 0.29 males (4/14) emigrated from trees with live fungi. One male migrated to the main study tree in 1985, while none was observed to emigrate from this population.

Fitness Components

The mean lifespan of the 67 males during the 1984 and 1985 mating seasons (including the truncated lifespans) is listed in the first line of Table 1 (W_1). The mean lifespan for 1984–1986 (including the eight truncated lifespans) is given in the second line of Table 1 (W_1'). The estimate of the true mean

TABLE 2. Statistics of the phenotypic characters (N = 67 males) in this study before selection. A) Means and standard deviations. B) Phenotypic correlations among the characters. All coefficients are significant at $P < 0.001$.

A. Character	Mean	SD
Elytral length (mm)	6.4	0.5
Horn length (mm)	2.0	0.9
Live weight (g)	0.10	0.02

B.	Character	
Character	Horn length	Elytral length
Elytral length	0.91	—
Live weight	0.89	0.85

lifespan of the entire cohort, taking the eight truncated lifespans into account, is listed in the third line of Table 1 (W_1''). Note the overlapping standard errors of these last two estimates; this suggests that the 1984–1986 estimate (W_1') is not affected much by the truncated observations. Also note that the 1984–1985 mean lifespan estimate (W_1) is 81% of the estimated total lifespan. The possible effects of using this underestimate of lifespan on the measures of selection will be discussed. Given the migration results above, it is not likely that these estimates were affected greatly by migration; however, possible effects of migration on the selection estimates will also be discussed.

The mean fitnesses for the sexual selection components (Table 1) show that, on average, males attended the mating area on about two-thirds of the nights that they were alive (W_2) and courted about one female per night that they were at the mating area (W_3). Sixty percent of these courtships actually ended in a copulation attempt (W_4), and 24% of these attempts were accepted by females (W_5). The last two lines of Table 1 show that males inseminate one female every 10 days of their lives (W_{2-5}), and the average male inseminates just over five females in his lifetime.

Selection on Phenotypic Traits

Selection Gradients.—The three phenotypic traits measured (weight, elytral length, and horn length) were positively correlated with each other (Table 2). Selection gradients estimate the direct selection acting on each of a suite of correlated characters and are therefore an appropriate measure of selection for these characters. The directional selection gradients with their standard errors for the five fitness components are given in Table 3. Note that the results for lifespan using the data through 1986 (W_1') are virtually indistinguishable from the results from the main study period only (W_1). This suggests that the data from the main study period accurately represent selection acting over the lifetimes of these males. The total r^2 values show that the three phenotypic characters together explained significant proportions of the variance in the third fitness component (number of females courted) and in the total sexual-selection component (components 2–5 combined). About 21% of the variance in total fitness was explained by the three characters. The only character under significant direct selection was horn length. The total fitness gradient of 0.94 indicates that, with other characters equal, males with horn lengths one phenotypic standard deviation greater than the population mean inseminated an average of 94% more females than the population mean insemination success. This selection for longer horns was principally sexual selection, with the last three components (3–5) being about equally important. None of the selection gradients for elytral length or weight was statistically significant.

The selection gradients for all 134 males present during the 1984 and 1985 mating seasons are given in Table 4. Note the strong similarity between these data and those for the cohort of 67 males in Table 3; since the sample size is doubled in Table 4, more of the coefficients and r^2 values are significant. The gradients for the first (lifespan) component of fitness and that for total fitness indicate that there is selection for shorter elytra that is principally due to natural (not sexual) selection. As in Table 3, the selection on horn length is significantly positive, although somewhat weaker. The differences between the coefficients in Tables 3 and 4 are not significant, either statistically or biologically.

Selection Differentials.—Standardized selection differentials, which measure total directional selection (both direct and indirect) on a character, are given in Table 5 for

TABLE 3. Standardized selection gradients (with standard errors) for the three phenotypic characters measured. Gradients were calculated by a multiple regression of relative fitness on the three characters. Each line of the table gives the results from a separate multiple regression, one line for each of the five components of fitness, one for lifespan including data from 1986, one for total sexual selection (components 2–5 combined or inseminations per night that the male was alive), and one for total fitness (the total number of females inseminated in a male's lifetime). $N = 67$ males for all rows except W_4 ($N = 62$) and W_5 ($N = 58$). Gradients for the five components that multiply to equal total fitness are in boldface. Significance levels of the total r^2 are from the F ratio (regression MS/residual MS). Significance levels for the gradients (the regression coefficients for each character) are from the t ratio (coefficient/standard error of coefficient).

Symbol	Fitness component	Characters: Elytral length	Horn length	Live weight	Total r^2
W_1	**Lifespan**	**−0.24** (0.18)	**0.26** (0.21)	**0.12** (0.17)	8.5
W_1'	Lifespan including 1986	−0.22 (0.21)	0.30 (0.24)	0.10 (0.19)	8.7
W_2	**Attendance/lifespan**	**0.04** (0.09)	**0.03** (0.10)	**−0.03** (0.08)	3.6
W_3	**Courtships/attendance**	**−0.01** (0.13)	**0.19** (0.15)	**−0.01** (0.12)	13.8*
W_4	**Copulation attempts/courtships**	**−0.06** (0.14)	**0.30*** (0.15)	**−0.20** (0.11)	8.8
W_5	**Inseminations/copulations**	**−0.05** (0.32)	**0.26** (0.33)	**0.00** (0.25)	6.0
W_{2-5}	Sexual selection (inseminations/lifespan)	−0.09 (0.23)	0.59* (0.26)	−0.06 (0.21)	27.3***
	Total fitness (number of inseminations)	−0.33 (0.32)	0.94** (0.36)	−0.16 (0.29)	20.7**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

the cohort of 67 males. The selection differentials for total fitness are significant and positive for all characters. For example, the total fitness differential of 0.49 for horn length means that selection increased the population mean horn length by about half of a standard deviation, or from 2.0 mm to 2.45 mm. The differential is less than the direct selection gradient (0.94), due to the negative direct selection on elytral length and weight, which are positively correlated with horn length [Table 2; Equation (1) shows how direct selection on correlated characters combine to produce total selection]. Note that total selection increased elytral length and weight, despite the negative direct selection (Table 3) on each of these characters. Indirect selection accounts for this apparent contradiction; the strong positive direct selection on horn length (Table 3), coupled with the large positive correlations between horn length and the other two characters, resulted in increases in elytral length and live weight. The total selection on elytral length and on weight, however, is slightly weaker than that on horn length. This is due to the weak negative direct selection on these two characters and the fact that the correlation between horn length and the other characters is less than 1.

DISCUSSION

This study shows how direct and indirect selection on correlated characters interact to produce the total selection on a given character. The high correlations among the characters measured in this study suggest that all three traits are indicators of general

TABLE 4. Standardized selection gradients (with standard errors) for all 134 males that were present at some time during the 1984 and 1985 mating seasons. For explanation, see Table 3.

Symbol	Fitness component	Characters: Elytral length	Horn length	Live weight	Total r^2
W_1	**Lifespan**	**−0.37*** (0.10)**	**0.23** (0.14)	**0.19** (0.11)	11.6**
W_2	**Attendance/lifespan**	**−0.08** (0.05)	**0.07** (0.07)	**0.06** (0.05)	6.5*
W_3	**Courtships/attendance**	**−0.07** (0.08)	**0.12** (0.10)	**0.07** (0.08)	8.5**
W_4	**Copulation attempts/courtships**	**−0.07** (0.07)	**0.17** (0.09)	**0.01** (0.07)	8.7**
W_5	**Inseminations/copulations**	**0.18** (0.14)	**0.03** (0.18)	**0.00** (0.14)	7.5*
W_{2-5}	Sexual selection (inseminations/lifespan)	−0.05 (0.12)	0.32* (0.16)	0.11 (0.12)	23.4***
	Total fitness (number of inseminations)	−0.47** (0.18)	0.62** (0.23)	0.22 (0.18)	18.7***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 5. Standardized selection differentials, which measure the total selection acting on the three phenotypic characters. Differentials are the slope of a univariate regression of relative fitness on each character individually. In all cases, the standard errors of the three differentials within each fitness component were the same, so they are given in one column at the end of each line. Sample sizes equal 67 males for all rows except W_4 ($N = 62$) and W_5 ($N = 58$). For explanation of the fitness components, see Table 3.

Symbol	Fitness component	Characters			SE
		Elytral length	Horn length	Live weight	
W_1	**Lifespan**	**0.10**	**0.15***	**0.14**	0.07
W_2	**Attendance/lifespan**	**0.05**	**0.05**	**0.04**	0.04
W_3	**Courtships/attendance**	**0.15****	**0.17****	**0.15****	0.05
W_4	**Copulation attempts/courtships**	**0.04**	**0.07**	**0.01**	0.05
W_5	**Inseminations/copulations**	**0.19**	**0.22**	**0.18**	0.11
W_{2-5}	Sexual selection (inseminations/lifespan)	0.39***	0.45***	0.38***	0.09
	Total fitness (number of inseminations)	0.38**	0.49***	0.39**	0.13

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

body size. The fact that the selection gradient for horn length differs in sign from the other two, however, suggests that there is selection for increased horn length independent of body size. Elytral length and live weight are probably both good indicators of body size; thus, selection gradients for these characters may reflect direct selection for body size, at least in part. The nonsignificant gradients for these two characters in the cohort (Table 3) do not indicate that either character is a target of selection; the analysis of the expanded data set (Table 4) suggests that elytral length may be slightly larger than optimal in terms of lifespan. The positive selection differentials on these characters, however, show that there is overall selection for increased adult body size due to the selection on horn size.

Most other studies that have partitioned fitness have used variance in relative fitness or the "opportunity for selection" as the only measure of selection (e.g., McCauley, 1983; Banks and Thompson, 1985; Fincke, 1986; Hafernik and Garrison, 1986; but see Arnold and Wade [1984*b*] and Koenig and Albano [1987]). Fitness variance is necessary for selection to occur, but variance alone is not sufficient evidence that selection has occurred. This is because fitness variance provides no information on the relationship between fitness and phenotypic characters and, consequently, does not measure selection acting on the phenotype. A large proportion of fitness variance may be random with respect to the phenotype (Sutherland, 1985*a*, 1985*b*); therefore, there can be great variance in fitness with little selection occurring.

Another possible use of the opportunity for selection is to help explain an observed lack of selection; however, similar information is available directly from a multiple regression analysis without calculating the opportunity for selection. There are two possible biological reasons for a lack of selection on a character. First, there may be ample fitness variance, but the relationship between fitness and the phenotype may be mostly random (Fig. 1A). Second, the variance in fitness may be very small (Fig. 1B); since the variance in fitness sets an upper bound on the amount a character can be changed by selection (Arnold and Wade, 1984*a*; Houck et al., 1985; Arnold, 1986), the lack of fitness variance explains the lack of selection in Figure 1B. One can distinguish between the two possible reasons for lack of selection using the r^2 value for the regression. The regression slopes in Figure 1A and 1B are the same (0.034)—that is, selection is weak in both examples. The r^2 values of the two regressions differ greatly, however; since there is little variance in Figure 1B more of this variance is explained by the same slope. Therefore, if the results of a regression analysis indicate that the slope(s) are small but the r^2 value is large, then this suggests that selection may have been limited by a lack of fitness variance. Conversely, if the r^2 value is small, then selection could not have been limited.

The r^2 values presented in this paper clearly indicate that selection on the three

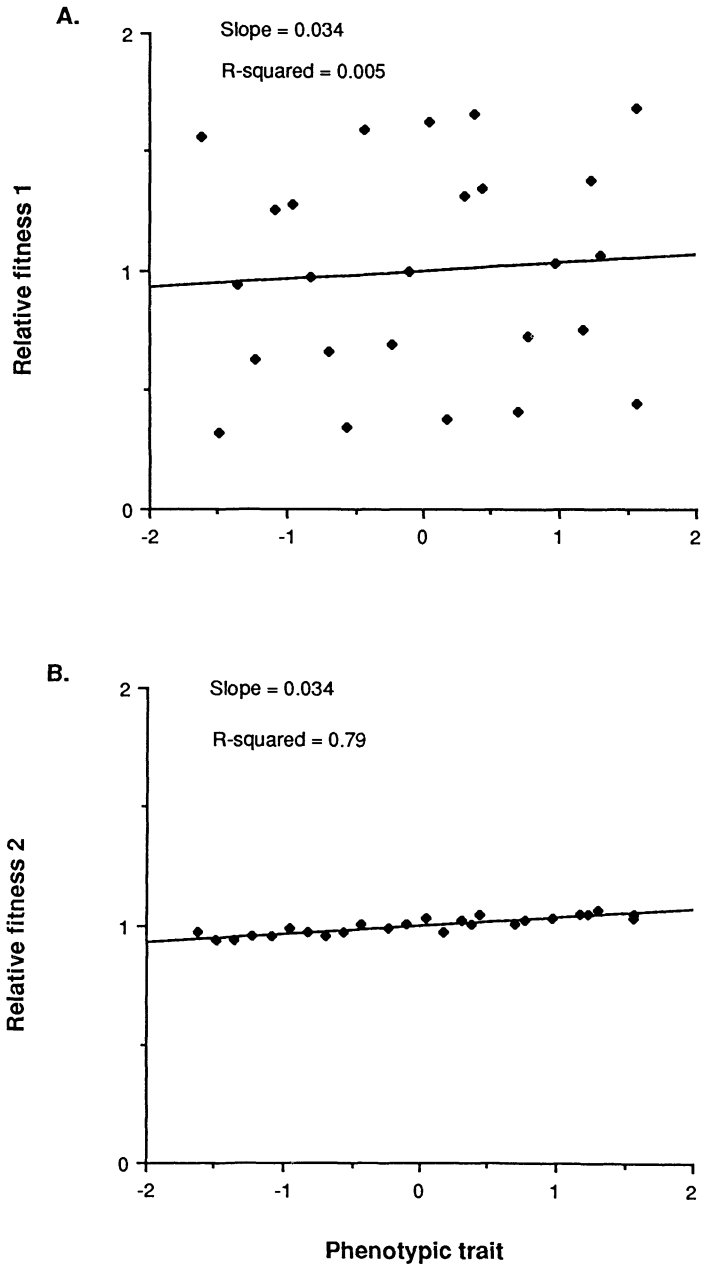

FIG. 1. Plots of relative fitness versus a phenotypic trait for two hypothetical populations, designed to illustrate the two possible reasons for a lack of selection on a character. The populations differ only in the variance in relative fitness; the mean relative fitness and the mean and variance of the phenotypic trait are the same in both populations. Note that selection is weak in both populations (both regression slopes equal 0.034), but the greater r^2 value in (B) suggests that selection may have been limited by a lack of fitness variance.

traits measured was not constrained by a lack of variance in any component of fitness. The three characters measured in this study together explained only about 21% of the variance in total fitness. What accounts for the other 79% of the fitness variance? First, there are obviously many characters that influence fitness other than the three mea-

sured. For example, behavioral traits could be important; characteristics of the males' long and complex (for a beetle) courtship behavior could well influence the percentage of a male's copulation attempts that are accepted by a female (W_5). Second, much of the variation in fitness in the five components may be random with respect to the phenotype.

Selective Agents and Targets of Selection

The results reported in this study illustrate how partitioning lifetime fitness into its components (Arnold and Wade, 1984*a*, 1984*b*) can separate the effects of natural and sexual selection on phenotypic characters and help identify the selective agents that are important (see Banks and Thompson [1985] and Koenig and Albano [1986] for discussions of this problem). For the expanded data set (but not for the cohort data alone) the gradients for the lifespan component indicated that there was selection for shorter elytra. Since this study did not deal with sources of mortality, the agents of selection can only be speculated upon. Perhaps males with longer elytra were more visible to predators. *B. cornutus* has three separate defense mechanisms that are adapted to different types of predators (Conner et al., 1985), suggesting that predation has been important in the evolutionary history of this beetle. The alternative explanation of increased metabolic costs of larger size is not likely, because the direct selection on weight was positive.

Most of the selection for longer horns, however, clearly involves sexual selection, and most of this is due to male competition for access to females. Recall that access to females (components 2–4) is determined principally by male competition and male choice. One would expect this result for several reasons: horns are a sexually dimorphic trait, horns are used in fights over females (Conner, 1987), and laboratory studies of *B. cornutus* (Brown, 1980; Brown and Siegfried, 1983; Brown et al., 1985; Brown and Bartalon, 1986) and other horned beetles (Eberhard, 1977, 1979, 1981; Palmer, 1978) also suggest that beetle horns have evolved for use in male competition.

Evolution of Phenotypic Traits

Based on the large positive selection differentials for all the characters found in this study (Table 5), one might predict that male *B. cornutus* are evolving longer horns and larger body sizes. The accuracy of this prediction, however, depends on two factors not addressed in this study. The first is the genetics of the traits. Clearly, if horn and body size are not heritable in this population, then there would be no response to the selection found in this study, and there would be no increase in size across generations. Results from laboratory studies of other species of insects suggest that body size is generally heritable (e.g., Bell and Burris, 1973; Palmer and Dingle, 1986), but these estimates are not directly applicable to *B. cornutus*. A related question involves the degree to which the phenotypic correlation between horn length and body size is caused by a genetic correlation (which depends in part upon the degree to which the two characters are heritable [Falconer, 1981]). If there is a significant positive genetic correlation, then the population will evolve larger bodies as a correlated response to the selection for longer horns, as is suggested by the selection differentials (Table 5).

The other important factor in predicting the evolution of these traits is the extent to which the selection differentials presented in this paper reflect the total selection acting on these traits. The first possible problem is the truncated lifespans and their effect on the selection differentials. It seems clear that the truncated lifespans do not affect the selection estimates for the lifespan component, since there is no difference between the gradients for the main data set and the set that includes 1986 data. The estimates of sexual selection presented here are valid unless the mating rate (i.e., inseminations per day) of the 17 surviving males changed greatly in 1986.

The second potential problem is the effect of migration on the estimates of lifespan. This is probably not a major problem, since only one male migrated from the study tree to one of the other 17 trees in 1985. It is possible that undetected migration oc-

curred, either within the woodlot studied or between the study tree and trees outside this patch of woods. It is unlikely that undetected migration occurred in large enough numbers to affect the results significantly, however, since most migrations were between trees that were less than 25 m apart. The distance from the study tree to the closest fungus-infested tree was more than 200 m, and the nearest woods outside the study patch was more than 500 m away. It is possible that there was greater migration in 1984 due to the warmer temperatures that year. This is unlikely to be a major problem for two reasons. First, if large numbers of beetles emigrated from the study tree in 1984, many of them would have been found at the other trees in 1985; only one 1984 emigrant was found. Second, in a five-year study of *B. cornutus* migration in a five-hectare study area in Virginia, L. Brown (pers. comm.) also found that migrations of more than 100 meters were extremely rare.

Another possibility is that *B. cornutus* tends to emigrate immediately after pupation, so that adults do not appear on the fungi until after migration. Given the intensity of observation and censusing at the study site, this seems unlikely, but it cannot be ruled out. If this early migration does occur, then selection due to differential migration success, which was not measured in this study, could be important. This would not affect the measurements of lifetime mating success within the main study population, however, because an immigrant's reproductive life would not begin until it appears on the fungi. Thus, it is likely that the beetle's first appearance on the tree is close to the beginning of its reproductive life. It is also likely that the last observation of a beetle accurately represents the beetle's death and not emigration, since males were much more likely to leave a tree after all fungi died (all 15 fungi on the study tree were alive after censuses were terminated).

The third potential problem is that this study only estimates selection due to variability in insemination success of adult males. Therefore selection on larvae and females has not been measured, nor has selection due to variance in male fecundity (eggs fathered per insemination). What are the likely effects of this unmeasured selection on the three phenotypic characters? The character under the strongest direct selection in this study was horn length. The selection gradients for horn length presented here probably represent all or most of the direct selection on horn length, because larvae and females do not have horns and male horn length is not likely to affect male fecundity directly, unless females choose to lay more eggs after mating with large-horned males.

If male *B. cornutus* with larger bodies have more offspring per insemination, then this could lessen the negative direct selection on elytra length or create positive selection on weight. In this case the positive total selection found on all three characters in this study would be an underestimate. A laboratory study of a cerambycid beetle showed that females produced more eggs per copulation when they were mated to larger males than when they were mated to smaller males (Hughes and Hughes, 1985).

If elytra length and weight in males are genetically correlated with the same characters in females, then selection on these characters in females would cause a correlated response to selection in males. Since males and females do not differ in elytral length (Conner, unpubl.), it seems reasonable to expect selection for shorter elytra in the lifespan component in females, as was found in males (Tables 3, 4). This selection for smaller bodies would not be opposed by selection for longer horns; however, there could be selection for larger-bodied females if fecundity is correlated with size in *B. cornutus* as it is in other insects (e.g., Parker, 1970; Juliano, 1985).

There can be no direct selection on horn length, elytra length, or adult weight at the larval stage, since these characters are not expressed in larvae. However, indirect selection due to selection on correlated characters in larvae (e.g., development time, growth rate, etc.) could be very important. For example, selection for shorter developmental times to reduce the risk of larval mortality would lead to smaller adult size, given a fixed larval growth rate. Thus, there could be directional selection for smaller size at the larval stage opposing the selection

for larger adult size. This directional selection in opposite directions at different life stages could give rise to overall stabilizing selection for body size (Endler, 1986; J. Travis, pers. comm.).

Finally, to understand the evolution of these traits in *B. cornutus,* temporal and spatial heterogeneity of selection must be considered. While this study covered two years, longer-term temporal fluctuations in selection are possible (e.g., selection due to differential migration ability). This study suggests that there is little emigration while the fungi are still alive, and since individual *G. applanatum* can live as long as 10 years (White, 1919), most individuals probably remain on the same tree throughout their lives. If success in colonization of new fungus-infested trees is related to horn length, elytral length, or weight, then this would cause periodic selection on these characters. In his five-year study of migration, however, L. Brown (pers. comm.) found no relationship between horn or elytral length and successful migration within a 5-hectare study site.

This study does not address interpopulational (spatial) differences in selection, since only one population was studied. In a study of two other lower-density populations, however, even stronger selection on male horn size was found (Conner, 1987), suggesting that the selection gradients reported here may underestimate selection on horn length over all populations of *B. cornutus.*

In summary, it seems clear that there is strong selection for longer horns in adult male *B. cornutus* and that this selection is not likely to be changed much by selection on females or by spatial and temporal variation in selection. It seems quite likely, however, that this selection might be opposed by negative selection on correlated characters in larvae. Whether or not this species is in the process of evolving longer horns depends upon the magnitude of this opposing indirect selection and the extent to which horn length is heritable.

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