

FEMALE LIFE SPAN AND FERTILITY ARE INCREASED BY THE EJACULATES OF PREFERRED MALES

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Abstract.—In animals with internal fertilization, sperm competition among males can favor the evolution of male ejaculate traits that are detrimental to females. Female mating preferences, in contrast, often favor traits in males that are beneficial to females, yet little is known about the effect of these preferences on the evolution of male ejaculates. A necessary condition for female preferences to affect the evolution of male ejaculate characteristics is that females select mates based on a trait correlated with ejaculate quality. Previous work has shown that females of the variable field cricket, *Gryllus lineaticeps*, prefer males that produce calling songs containing faster and longer chirps. In this study, we tested the hypothesis that females receive more beneficial ejaculates from preferred males. Females were placed on either a high- or a reduced-nutrition diet then mated twice to a male of known song phenotype. Females received only sperm and seminal fluid from males during these matings. There was no effect of male song phenotype on any fitness component for females on the high-nutrition diet. Reduced-nutrition females mated to males that produced preferred song types, however, lived longer, produced more eggs, produced more fertile eggs, and had a higher proportion of their eggs fertilized than those mated to other males. The life-span benefit was positively associated with male chirp duration, and the reproductive benefits were positively associated with male chirp rate. We explored two possible mechanisms for the life span and reproductive benefits. First, a path analysis suggested that part of the effect of male chirp duration on female life span may have been indirect; females mated to males that produced longer chirps showed delayed oviposition, and females that delayed oviposition lived longer. Males that produce longer chirps may thus transfer fewer or less potent oviposition stimulants to females in their seminal fluid. Second, there was a positive correlation between male chirp rate and the number of sperm transferred to females. The fertility benefit may thus have resulted from females receiving more sperm from males that produce faster chirps. Finally, there was a negative phenotypic correlation between male chirp rate and chirp duration, suggesting that females may have to trade off the life span and reproduction benefits when selecting a mate.

Key words.—Calling song, direct benefits, female preference, field cricket, *Gryllus lineaticeps*, male ejaculate, sperm.

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Female animals often choose among potential mates based on variation in male mating signals. Such preferences commonly evolve because females benefit from mating with males with preferred phenotypes (e.g., Knapp and Kovach 1991; Petrie 1994). Female choice based on a signal correlated with the quality of a mating benefit is possible only if the male signal provides reliable information about the quality of the benefit. But deceptive signaling by males of either low quality, which may advertise a benefit they cannot provide, or males of high quality, which may advertise a benefit they could but will not provide, can compromise signal reliability. There are two general conditions that are thought to constrain deceptive signaling. First, a given increase in signal value may have greater marginal fitness costs for males that are unable to provide high-quality benefits to females (Zahavi 1975; Nur and Hasson 1984; Grafen 1990; Iwasa et al. 1991; Price et al. 1993). Second, a given increase in signal value may have greater marginal fitness benefits for males that provide higher quality benefits to females (Getty 1998). When either of these conditions is met, the optimal signal value for males of higher quality will be greater than that for males of lower quality, and reliable signaling of mating benefits will be maintained. When females choose mates based on signals that are reliably correlated with mating benefits, sexual selection through female choice may affect the evolution of both male signals and the quality of the benefits provided by males.

Females can receive a variety of benefits from mating with preferred males, including material benefits that affect female

fitness, material benefits that affect offspring fitness, and genetic benefits that affect offspring fitness (reviewed by Andersson 1994). When females receive only ejaculates from males, it is commonly assumed that any benefit of mate choice must be due to an increase in offspring fitness that results from mating with genetically superior males. In animals with internal fertilization, however, females receive not only genes but also sperm and a variety of seminal fluid products in male ejaculates. The quantity and quality of sperm received from a male can affect the number of fertile eggs that a female produces (Levitan and Peterson 1995; Matthews et al. 1997; Drnevich 2001), while male seminal fluid products can affect female life span and reproduction (Ridley 1988; Arnqvist and Nilsson 2000; Wagner et al. 2001a). As a result, females may directly benefit from mating with preferred males if ejaculate quality covaries with a trait used by females in mate choice. Females may also benefit if male products are incorporated into eggs and affect offspring fitness (e.g., Dussourd et al. 1989; Iyengar and Eisner 1999) and if the quality of these products covaries with a trait used in mate choice. If male signals covary with ejaculate quality, and if females select mates based on these signals, males that transfer higher quality ejaculates will mate more frequently. Female choice should thus affect the evolution of male ejaculates as long as there are mechanisms that disfavor deceptive signaling of ejaculate quality.

While female choice may be an important source of selection on male ejaculate characteristics, research on the evolution of male ejaculates has tended to focus on the effects

of sperm competition. Sperm competition favors traits in males that increase fertilization success when the sperm of two or more males are in competition for a given set of eggs. Some of the traits favored by sperm competition, including greater sperm quantity and larger sperm size (reviewed in Birkhead and Møller 1998), may have few deleterious effects on female fitness. Other traits, however, such as a variety of seminal fluid products transferred by males in species with internal fertilization, can be detrimental to the females within whose bodies sperm competition occurs (reviewed by Stockley 1997; Simmons and Siva-Jothy 1998). Because these male traits can result in mating costs for females, there may often be conflicts of interest between males and females that can have important consequences for the dynamics of male-female coevolution (Parker 1979, 1984). For example, in the fruit fly *Drosophila melanogaster*, males transfer toxic peptides to females in their seminal fluid; while these peptides appear to increase a male's success in sperm competition (Harshman and Prout 1994; Clarke et al. 1995), they reduce female life span (Fowler and Partridge 1989; Chapman et al. 1995). The transfer of harmful seminal fluid products can lead to antagonistic coevolution between the sexes, in which males continually evolve products that increase their success in sperm competition and females evolve behavioral or physiological mechanisms to reduce the costs of these products (Rice 1996; Holland and Rice 1998, 1999). The nature of the coevolutionary interaction between males and females may thus differ substantially depending on whether female choice or sperm competition has a larger effect on the evolution of male ejaculates.

In the variable field cricket, *Gryllus lineaticeps* (Orthoptera, Gryllidae), males produce a calling song to attract females that consists of a series of short, rapidly repeated chirps, and females prefer calling songs with higher chirp rates and longer chirp durations (Wagner 1996; Wagner and Reiser 2000; Wagner et al. 2001b). Females that mate more frequently live longer, even when all they receive from males is sperm and seminal fluid, suggesting that females may receive beneficial rather than detrimental ejaculate products from males during mating (Wagner et al. 2001a). In this study, we tested the hypothesis that females receive direct benefits from mating with males that produce preferred calling song because of correlated variation in male ejaculate quality. If females select mates based on variation in ejaculate quality, there is the potential for female choice to affect the evolution of male ejaculates.

MATERIALS AND METHODS

General Methods

The animals used were the first- or second-generation offspring of field-inseminated females collected from Tucker's Grove Park, Santa Barbara, California. We propagated the crickets by arranging matings between individuals of known ancestry. Nymphs were raised in family containers until the final instar, at which time we transferred them to individual containers. All crickets were thus known to be virgins at the beginning of the experiments. The individual containers were checked daily so that we could determine the day on which the crickets reached sexual maturity. Crickets were

provided with ad libitum water and cat chow. Males were maintained on this diet throughout their lives. Depending upon the study, females were either maintained on this diet throughout their lives or placed on one of two feeding regimes 6 days after adult eclosion (see below).

Male Song and Female Fitness

To test the hypothesis that females benefit from the ejaculates of preferred males, we mated females to males that varied in singing behavior and then measured female life span, the total number of eggs that a female produced, the total number of fertile eggs that a female produced, and the proportion of a female's eggs that were fertilized.

The calling songs of virgin adult males were recorded 6 to 11 days after adult eclosion ($\bar{x} = 7.75$, $SE = 0.14$). Male songs were recorded and analyzed using a Cambridge Electronic Design (Cambridge, U.K.) Micro1401 sound acquisition interface, Spike2 software (Cambridge Electronic Design) and a Macintosh (Cupertino, CA) computer. Males were recorded in individual semi-anechoic chambers equipped with a Sennheiser (Wedemark, Germany) ME 67 microphone with a K6 power module connected to the Micro1401. Because the duration of singing activity varied among males, the segment of continuous calling song that we analyzed ranged between 30 and 180 sec. Songs were digitized at a sampling rate of 22 kHz, and they were analyzed using a custom-designed script that automatically calculated chirp rate and average chirp duration. Chirp rate (CR; chirps/sec) was measured as the number of chirps a male produced over the sampling interval divided by the duration of the sampling interval. Chirp duration (CD; msec) was measured as the average duration of all chirps produced during the sampling interval. Both of these song characters are affected by temperature (Hoback and Wagner 1997; Wagner and Reiser 2000). Because comparisons of male song phenotypes are potentially confounded by the effect of recording temperature on singing behavior, we adjusted all song characters to the mean recording temperature of 24.3°C ($SE = 0.1$) prior to statistical analysis based on the regression of each song character on temperature as follows: $CR_{adj} = CR_{obs} + 0.034(24.3 - TEMP_{obs})$; $CD_{adj} = CD_{obs} - 0.004(24.3 - TEMP_{obs})$. None of the analyses were affected by the use of temperature-adjusted song characters.

We mated each male twice to a virgin female. Males and females were randomly paired with the restrictions that the male had been recorded within 5 days of the first mating ($\bar{x} = 1.80$ days, $SE = 0.18$) and that the male and female within a pair were neither full- nor half-siblings. The matings for each female occurred 10 and 24 days following adult eclosion, and each male and female was weighed to the nearest 0.0001 g prior to the first mating. We mated females more than once to increase our chances of detecting an effect of male phenotype on female fitness (i.e., to magnify differences among males in the quantity of ejaculate products). Females were mated only twice because of the time required to conduct the matings. Male products may affect female fitness early in life, late in life, or during both time periods. We mated females both earlier and later in life so that we had the ability to detect either type of effect.

During the experimental pairings we observed the pairs until a mating occurred, after which the male was removed and housed in an individual container. During mating male field crickets transfer a spermatophore to females that consists of an outer covering and an internal ampulla that contains sperm and seminal fluid. This spermatophore is attached externally to a female's genital pore, and sperm and seminal fluid enter the female's reproductive tract through a spermatophore tube that the male inserts into the female reproductive tract. Approximately 30–90 min after attachment, the female usually removes and consumes the spermatophore (Wagner et al. 2001a). Female insects are known to receive nutrients from spermatophore consumption, and in those species where the spermatophore is deposited inside the female's reproductive tract, from the breakdown of the spermatophore within the reproductive tract (reviewed by Boggs 1995; Gwynne 1997; Vahed 1998). Females can also receive hydration benefits from spermatophore consumption (Ivy et al. 1999). Because we were interested in the effect of male ejaculates on female fitness, independent of any effects of spermatophore consumption, we removed and discarded all spermatophores 30 min after attachment in the experimental matings. As a result, females only received sperm and seminal fluid from males during these matings. After the first mating we housed females in individual plastic containers with food, water, and a shell vial filled with rolled and moistened cheesecloth for oviposition.

We collected eggs and checked females for mortality daily after the first mating. The eggs were incubated in moistened cheesecloth in a temperature-controlled chamber at 25°C. Beginning 8 days after collection and continuing until 20 days after collection, we assessed the eggs daily for evidence of development. If eyespots or segmentation was present, we scored the eggs as fertilized. If neither trait was present by day 20, we scored the eggs as unfertilized. We obtained four measures of female fitness: life span, lifetime fecundity, lifetime fertility, and the proportion of eggs fertilized. Life span was scored as the number of days a female survived after adult eclosion. Lifetime fecundity was scored as the total number of eggs produced by a female. Lifetime fertility was scored as the total number of eggs produced by a female that showed evidence of development. The proportion of eggs fertilized was calculated as: lifetime fertility/lifetime fecundity.

Because the abundance of available resources may affect whether females benefit from mating with preferred males, we placed females on either a high- or a reduced-nutrition diet six days after adult eclosion (diet described in Wagner and Hoback 1999; Wagner and Reiser 2000). Because smaller females require less food than larger females, we varied the amount of food provided to a female based on initial female mass. Prior to the start of the diet manipulation, we conducted a preliminary study using a separate group of females to examine the relationship between female mass and daily consumption of the experimental diet. Based on the results of this preliminary experiment, the amount of provided to a given female was the quantity of the high-nutrition diet that an average female of that mass would consume daily (daily food [g] = [female mass × 0.04577] – 0.01). All females of a given mass thus received the same quantity of food,

while heavier females received more food than lighter females. Females on the high-nutrition diet received their full mass-associated allotment of the experimental diet. Females on the reduced-nutrition diet received the same mass-associated allotment of food, but the experimental diet was cut with 50% non-nutritive cellulose. As a result, females on the reduced-nutrition diet received half as many nutrients as females on the high-nutrition diet. Because females were provided with a given quantity of food, females on the reduced-nutrition diet were unable to compensate for their low-quality diet by consuming more food. Females were maintained on the experimental diets until they died.

We examined the effect of male song phenotype on female life span, lifetime fecundity, lifetime fertility, and the proportion of eggs fertilized using a series of multiple regressions. Because female mass had a significant effect on a number of components of female fitness, female mass at the time of the first mating was included as an independent variable in all analyses. In addition, because male mass was positively correlated with chirp duration (see Results), male mass at the time of the first mating was included as an independent variable in all analyses. Male mass, however, did not have a significant effect on any component of female fitness. The data were thus reanalyzed excluding male mass. We only report the results of the latter analyses.

Trade-Off between Benefits

We examined the correlation between male chirp rate and chirp duration to determine if females potentially have to trade off benefits associated with these two song characters. Three data sets were used to examine the correlation between the calling song characters. First, we used the songs of males that were mated to females in the study of female mating benefits. Second, we used a larger laboratory sample of male calling song. The males used were the first- or second-generation offspring of field-inseminated females collected in Tucker's Grove County Park, Santa Barbara, California. A 2-min sample of calling song was recorded from 388 males using a Sony TCD-5M stereo cassette recorder and a Sennheiser ME-80 microphone with a K3-U power module. The recordings were analyzed on a Macintosh Quadra 840AV computer using Canary (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY) sound analysis software. Chirp rate was measured as the number of chirps produced over the 2-min sample divided by 120 sec (chirps/sec). Chirp duration was measured as the average duration of six randomly selected chirps (msec). Because chirp rate and chirp duration vary with temperature, the song data were adjusted to 24.3°C based on the regression of each trait on recording temperature. Third, we used a field sample of male calling song. A 2-min sample of calling song was recorded from 78 males in Tucker's Grove County Park. Data on the temperature at the position of a singing male was collected immediately following a recording. Songs were analyzed and adjusted for temperature as with the large laboratory sample of male calling song.

Male Song and Sperm Transfer

We examined the relationship between male singing behavior and the amount of sperm transferred by a male in its

TABLE 1. Comparison of fitness components between females on the high- and reduced-nutrition diets (high nutrition: $N = 33$; reduced nutrition: $N = 27$).

Variable	Diet treatment (mean \pm SE)		t	P
	High nutrition	Reduced nutrition		
Life span (days)	75.5 \pm 6.2	54.6 \pm 5.3	2.50	0.015
Lifetime fecundity	269.4 \pm 41.2	203.7 \pm 32.4	1.21	0.231
Lifetime fertility	129.3 \pm 22.5	129.2 \pm 25.1	0.00	0.997
Proportion of eggs fertilized	0.42 \pm 0.05	0.53 \pm 0.05	1.68	0.098

spermatophore to determine if an earlier discovered fertility benefit could be a result of females receiving more sperm from preferred males. Two-minute samples of the calling songs of 23 virgin males were recorded using a Sony TCD-5M stereo cassette recorder and a Sennheiser ME 67 microphone with a K6 power module. The songs were digitized at a sampling rate of 44.1 kHz using a Macintosh computer and they were analyzed using Canary sound analysis software. Chirp rate was measured as the number of chirps produced over the 2-min sample divided by 120 sec (chirps/sec). Chirp duration was measured as the average duration of six randomly selected chirps (msec). Chirp rate and duration were adjusted to 24.3°C prior to statistical analysis. After a recording, the male was placed with a single randomly selected female from our stocks. Immediately after the male transferred a spermatophore to the female, we removed the spermatophore with forceps. The spermatophore was placed in a microcentrifuge tube containing 5 ml of distilled water and crushed with forceps. The solution was repeatedly drawn into and expelled from a 1 ml tuberculin syringe to further break apart the spermatophore. The microcentrifuge tube was vortexed to prevent the sperm from agglutinating, then six 10- μ l samples were pipetted onto microscope slides. The slides were allowed to dry at room temperature for at least 30 min prior to counting. The number of sperm heads in the samples

was counted using a hand-drawn grid and a Meiji Techno RZ dissecting microscope at 500 \times magnification, and we calculated the average number of sperm cells per sample.

RESULTS

Effect of Diet on Female Fitness

Females on the high-nutrition diet lived significantly longer than females on the reduced-nutrition diet (Table 1). There were no significant effects of diet on lifetime fecundity, lifetime fertility, or the proportion of eggs that were fertilized (Table 1).

Male Singing Behavior

The males that were mated to females had temperature-adjusted chirp rates that varied between 1.0 and 3.8 chirps/sec ($N = 60$, $\bar{x} = 2.41$ chirps/sec, SE = 0.10) and temperature-adjusted chirp durations that varied between 75 and 137 msec ($N = 60$, $\bar{x} = 103.50$ msec, SE = 1.81). Chirp rate was not significantly correlated with male mass ($r_{58} = 0.24$, $P = 0.066$), although there was a trend toward larger males producing faster chirps. There was a significant positive correlation between chirp duration and male mass ($r_{58} = 0.29$, $P = 0.025$).

Male Song and Female Fitness: High-Nutrition Diet

We used multiple regression analyses to examine the effect of male phenotype and female mass on the fitness of females on the high-nutrition diet. Male mass did not have a significant independent effect on any female fitness component ($P > 0.187$ for the partial regression of male mass on all female fitness components). As a result, we removed male mass from the multiple regression analyses.

For females on the high-nutrition diet, there were no significant effects of male chirp rate or chirp duration on female life span, lifetime fecundity, lifetime fertility, or the proportion of eggs fertilized (Table 2). There were also no significant effects of female mass on these fitness components, although there was a marginally significant positive association between female mass and lifetime fecundity (Table 2).

Male Song and Female Fitness: Reduced-Nutrition Diet

We used multiple regression analyses to examine the effect of male phenotype and female mass on the fitness of females on the reduced-nutrition diet. Male mass did not have a significant independent effect on any female fitness component ($P > 0.108$ for the partial regression of male mass on all

TABLE 2. Multiple regression of male singing behavior, male mass, and female mass on the life span ($F_{3,29} = 0.98$, $P = 0.412$, $r^2 = 0.09$), lifetime fecundity ($F_{3,29} = 1.47$, $P = 0.244$, $r^2 = 0.13$), lifetime fertility ($F_{3,29} = 1.09$, $P = 0.369$, $r^2 = 0.10$), and proportion of eggs fertilized ($F_{3,29} = 0.30$, $P = 0.822$, $r^2 = 0.03$) for females maintained on a high-nutrition diet.

Variable	Coefficient	SE	t	P
Life span				
Male chirp rate	-10.73	9.53	1.13	0.269
Male chirp duration	-349.03	454.37	0.77	0.449
Female mass	62.60	45.52	1.38	0.180
Lifetime fecundity				
Male chirp rate	-19.26	62.13	0.31	0.759
Male chirp duration	-3708.79	2962.83	1.25	0.221
Female mass	552.39	296.80	1.86	0.073
Lifetime fertility				
Male chirp rate	-6.94	34.48	0.20	0.842
Male chirp duration	-2015.94	1644.21	1.23	0.230
Female mass	246.82	164.71	1.50	0.145
Proportion of eggs fertilized				
Male chirp rate	0.01	0.09	0.10	0.921
Male chirp duration	-3.53	4.06	0.87	0.392
Female mass	-0.05	0.41	0.14	0.891

TABLE 3. Multiple regression of male singing behavior, male mass, and female mass on the life span ($F_{3,23} = 6.85$, $P = 0.002$, $r^2 = 0.47$), lifetime fecundity ($F_{3,20} = 8.10$, $P < 0.001$, $r^2 = 0.51$), lifetime fertility ($F_{3,23} = 5.93$, $P = 0.004$, $r^2 = 0.44$), and proportion of eggs fertilized ($F_{3,23} = 1.91$, $P = 0.156$, $r^2 = 0.20$) for females maintained on a reduced-nutrition diet.

Variable	Coefficient	SE	<i>t</i>	<i>P</i>
Life span				
Male chirp rate	5.16	5.22	0.99	0.333
Male chirp duration	1133.43	314.18	3.61	0.002
Female mass	63.92	23.46	2.72	0.012
Lifetime fecundity				
Male chirp rate	62.87	30.43	2.36	0.050
Male chirp duration	1084.41	1832.44	0.59	0.560
Female mass	617.43	136.83	4.51	<0.001
Lifetime fertility				
Male chirp rate	62.23	25.43	2.45	0.023
Male chirp duration	1806.19	1531.34	1.18	0.250
Female mass	392.92	114.34	3.44	0.002
Proportion of eggs fertilized				
Male chirp rate	0.18	0.08	2.26	0.034
Male chirp duration	4.06	4.72	0.86	0.399
Female mass	0.29	0.35	0.81	0.425

female fitness components). As a result, we removed male mass from the multiple regression analyses.

There were significant effects of male song phenotype and female mass on female life span (Table 3). Reduced-nutrition females that were mated to males that produced longer chirps lived longer than females that were mated to males that produced shorter chirps (Fig. 1A). On average, a 1-SD increase in the chirp duration of a female's mate increased female life span by 15.8 days, a 28.9% increase over the average life span. There was not a significant effect of male chirp rate on female life span, but there was a significant positive effect of female mass on life span.

There were significant effects of male song phenotype and female mass on female lifetime fecundity (Table 3). Reduced-nutrition females that were mated to males that produced faster chirps produced more eggs than females that were mated to males that produced slower chirps. On average, a 1-SD increase in the chirp rate of a female's mate increased female lifetime fecundity by 52.8 eggs, a 19.6% increase over the average lifetime fecundity. There was not a significant effect of male chirp duration on female life span, but there was a significant positive effect of female mass on life span.

There were significant effects of male song phenotype and female mass on female lifetime fertility (Table 3). Reduced-nutrition females that were mated to males that produced faster chirps produced more fertile eggs than females that were mated to males that produced slower chirps (Fig. 1B). On average, a 1-SD increase in the chirp rate of a female's mate increased female lifetime fertility by 52.3 eggs, a 40.4% increase over the average lifetime fertility. There was not a significant effect of male chirp duration on female lifetime fertility, but there was a significant positive effect of female mass on lifetime fertility.

The increase in the fertility of reduced-nutrition females mated to males that produced faster chirps may have resulted, in part, from a positive effect of male chirp rate on the pro-

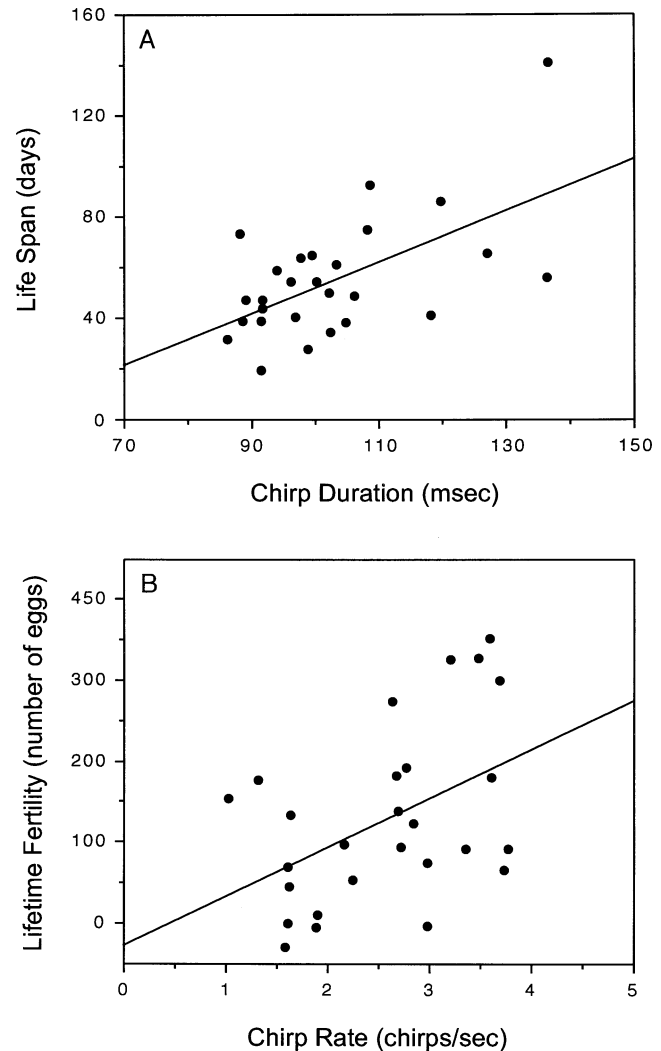


FIG. 1. Effect of male singing behavior on female life span and lifetime fertility. (A) Partial regression of male chirp duration on female life span ($N = 27$). The female mated to the male that produced the longest chirps lived substantially longer than all other females in the experiment. The relationship between male chirp duration and female life span is significant and positive even when this female is excluded from the analysis ($t_{22} = 2.22$, $P = 0.037$). (B) Partial regression of male chirp rate on female lifetime fertility ($N = 27$).

portion of a female's eggs that were fertilized (Table 3). Reduced-nutrition females that were mated to males that produced faster chirps produced a higher proportion of fertilized eggs than females that were mated to males that produced slower chirps. On average, a 1-SD increase in the chirp rate of a female's mate increased the proportion of fertilized eggs by 24.4%. There was not a significant effect of either male chirp duration or female mass on the proportion of eggs fertilized.

Male Phenotype, the Timing of Reproduction, and Female Life Span

To explore the mechanism through which male phenotype affected the life spans of females on the reduced-nutrition

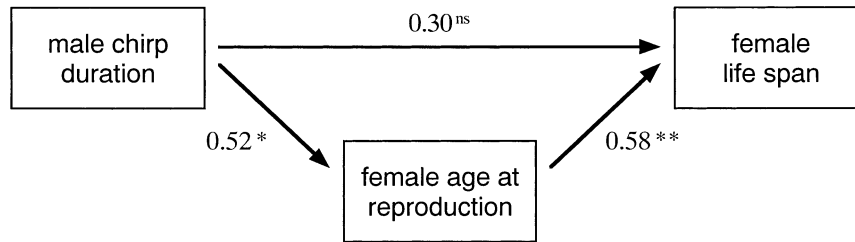


FIG. 2. Path diagram and path coefficients for the effect of male chirp duration on female life span. * $P < 0.05$, ** $P < 0.01$.

diet, we examined the pattern of correlation among male singing behavior, female age at first reproduction (defined as the number of days after the first mating to lay 20 fertilized eggs), and female life span. Females that never laid 20 fertilized eggs were not included in the analysis. Female life span was positively correlated with male chirp duration ($r_{19} = 0.60$, $P = 0.003$) but was not correlated with male chirp rate ($r_{19} = -0.08$, $P = 0.742$). Female life span was also positively correlated with age at first reproduction ($r_{19} = 0.73$, $P < 0.001$); females that began reproducing later lived longer. In addition, female age at first reproduction was positively correlated with male chirp duration ($r_{19} = 0.52$, $P < 0.014$) but was not correlated with male chirp rate ($r_{19} = -0.02$, $P = 0.943$); females that were mated to males that produced longer chirps showed a delay in oviposition. It is possible that male chirp duration and female age at first reproduction independently affected female life span. Alternatively, chirp duration may have indirectly affected female life span through an effect of chirp duration on the age at first reproduction.

We used path analysis to assess the direct and indirect effects of male chirp duration on female life span (Fig. 2). The path analysis is based on a multiple regression of male chirp duration and female age at first reproduction on female life span, a simple regression of male chirp duration on female age at first reproduction, and a simple regression of male chirp duration on female life span. A direct effect of chirp duration on female life span would suggest that males that produce longer chirps transfer products to females that directly increase female longevity. An indirect effect of male chirp duration on female life span would suggest that males that produce longer chirps transfer products to females that delay reproduction and that this delay in reproduction causes an increase in female longevity.

First, male chirp duration had a positive but nonsignificant direct effect on female life span when controlling for female age at reproduction ($t_{18} = 1.74$, $P = 0.098$, path coefficient [i.e., standardized regression coefficient] = 0.30). Second, male chirp duration had a significant direct effect on female age at first reproduction; females that were mated to males that produced longer chirps began reproducing at a later age ($t_{19} = 2.65$, $P = 0.016$, path coefficient = 0.52). Third, female age at first reproduction had a significant direct effect on life span when controlling for male chirp rate; females that began reproducing later lived longer ($t_{18} = 3.32$, $P = 0.004$, path coefficient = 0.58). The magnitude of the indirect effect of male chirp duration on female life span is the product of the latter two paths, which is 0.30, identical in magnitude to the direct effect. Although neither the direct nor the indirect effects

of chirp duration were significant by themselves, these effects together resulted in a significant total effect of male chirp duration on female life span ($t_{19} = 3.29$, $P = 0.004$, coefficient = 0.60).

Trade-Off between Benefits

Females may often have to trade off the benefits of mating with males that produce faster chirps and the benefits of mating with males that produce longer chirps. For the males that were mated to females in the benefits study, there was a marginally significant negative correlation between chirp rate and chirp duration ($r_{58} = -0.25$, $P = 0.059$). In a larger sample of male song recorded in the laboratory, there was a significant negative correlation between chirp rate and chirp duration ($r_{386} = -0.24$, $P < 0.001$; Fig. 3A). There was also a significant negative correlation between chirp rate and chirp duration in a sample of male song recorded in the field ($r_{76} = -0.43$, $P < 0.001$; Fig. 3B). Males that produce faster chirps thus tend to produce shorter chirps, whereas males that produce longer chirps tend to produce slower chirps.

Female Sperm Limitation

Females on both experimental diets appear to have been sperm limited; on average, only 46.9% of a female's eggs showed evidence of having been fertilized (Table 1; range: 0–92.5%). Sperm limitation should be reflected in a decline in the proportion of eggs fertilized over time; the greater the time since the last mating, the lower the proportion of eggs that a female should be able to fertilize because of diminishing sperm supplies. To examine the effect of time on fertilization success, we compared the proportion of eggs fertilized within the first 21 days of adult eclosion with the proportion of eggs fertilized more than 21 days after adult eclosion. In these comparisons, some females were excluded because they produced no eggs during one of the time periods. For females on both diet treatments, a greater proportion of early eggs were fertilized than later eggs (high-nutrition diet: paired- $t_{20} = 6.43$, $P < 0.001$; reduced-nutrition diet: paired- $t_{17} = 4.65$, $P < 0.001$; Fig. 4). Sperm limitation should also be reflected in the pattern of correlation among female fitness components. Across the two diet treatments, females that lived longer produced significantly more eggs ($r_{58} = 0.43$, $P < 0.001$) but did not produce significantly more fertilized eggs ($r_{58} = 0.18$, $P = 0.162$). As a result, there was a significant negative correlation between life span and the proportion of eggs fertilized ($r_{58} = -0.26$, $P = 0.042$). These results suggest that unless longer-lived females mate more than twice, they may deplete their sperm stores later in life

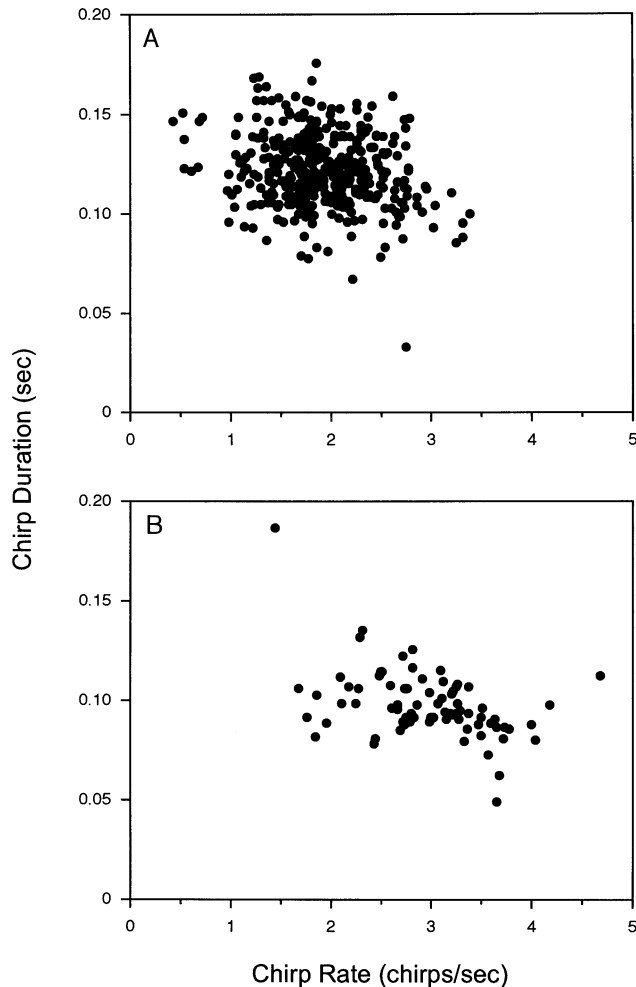


FIG. 3. Correlation between male chirp rate and chirp duration. (A) Laboratory recordings ($N = 388$). (B) Field recordings ($N = 78$).

and lay primarily unfertilized eggs. It is also possible, however, that older females produced a higher proportion of fertilized eggs that failed to develop.

Male Song and Sperm Transfer

To determine whether the female fertility benefit might result from females receiving more sperm from males that produce faster chirps, we used multiple regression analysis to examine the effect of male song phenotype and male size on the quantity of sperm contained in a male's spermatophore. Male phenotype had a significant effect on the number of sperm transferred in a male's spermatophore (multiple regression: $F_{3,19} = 3.22$, $P = 0.046$, $r^2 = 0.34$). Male sperm count was positively associated with chirp rate ($t_{19} = 2.87$, $P = 0.010$; Fig. 5), but there was not a significant association of sperm count with either chirp duration ($t_{19} = 0.90$, $P = 0.379$) or male mass ($t_{19} = 0.33$, $P = 0.746$).

DISCUSSION

Direct Benefits of Female Mating Preferences

Female *G. lineaticeps* prefer male calling songs with faster chirps and calling songs with longer chirps (Wagner 1996;

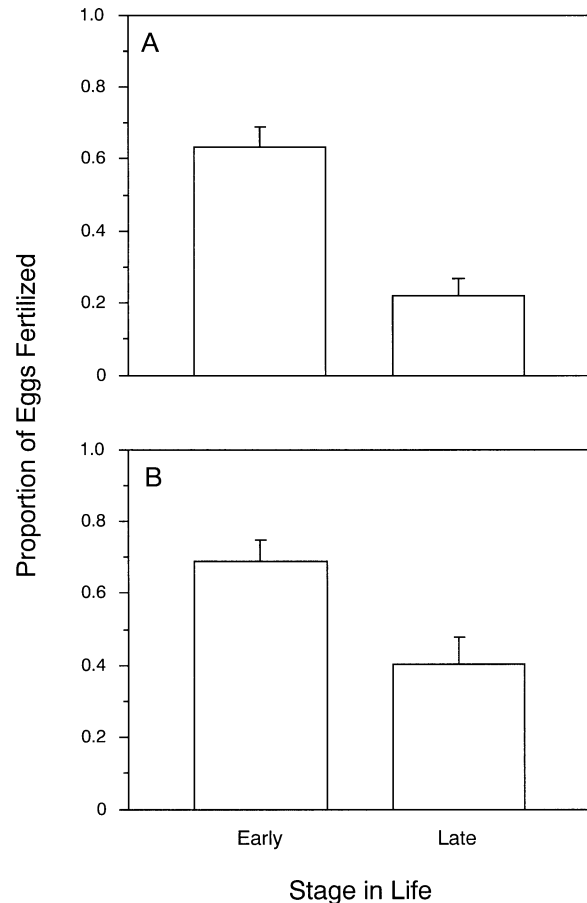


FIG. 4. The proportion of eggs laid by females early in life (within 21 days of adult eclosion) and late in life (more than 21 days after adult eclosion) that showed evidence of fertilization (mean \pm SE). (A) Females on the high-nutrition diet ($N = 21$). (B) Females on the reduced-nutrition diet ($N = 18$).

Wagner and Reiser 2000; Wagner et al. 2001b). The results presented here suggest that females that consume a low-quality diet may directly benefit from these mating preferences because of covariation between male singing behavior and male ejaculate characteristics. Reduced-nutrition females that were mated to males that produced longer chirps lived longer, and reduced-nutrition females that were mated to males that produced faster chirps had greater lifetime fecundity and fertility. In these matings, females were prevented from consuming spermatophores. As a result, all that females received from males was ejaculate. While singing behavior was correlated with mass in the males that were mated to females, an effect of mass on singing behavior was not seen in a field sample of singing males (Wagner and Hoback 1999), and there was no effect of male mass on female fitness in the current study. The correlations between male song characters and female fitness components therefore appear to be independent of male size. Genetic benefits are often invoked to explain female mating preferences in species where there is no obvious male contribution to females (i.e., species in which males provide no food gifts, no access to resources on a territory, and no parental care). In such species, it is commonly stated, "females receive only sperm from males." But

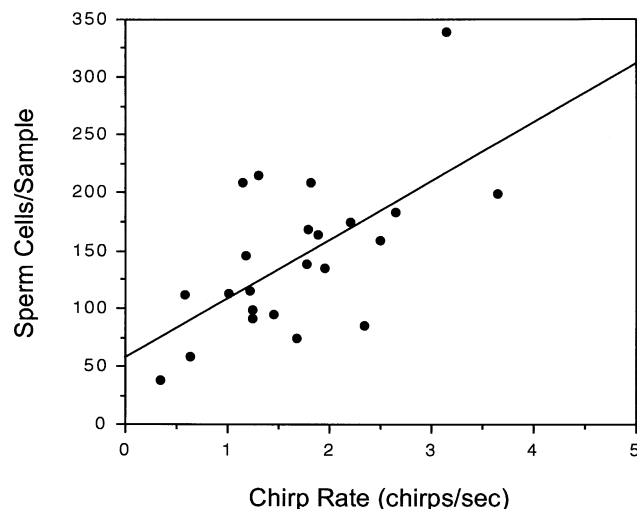


FIG. 5. Partial regression of male chirp rate on the average number of sperm cells per sample from a male's spermatophore ($N = 23$).

male ejaculates contain both sperm and seminal fluid, both of which can vary in quantity and quality. As our results illustrate, females may benefit directly from their mating preferences even when they only receive ejaculates from their mates.

Reproductive Benefits

Females can obtain fertility benefits from their mates if they are occasionally sperm limited and if male phenotype covaries with the quantity or quality of sperm transferred to a female. Under such conditions, female mating preferences may reduce the risk of sperm limitation. A large body of research has shown that female fertility in arthropods often increases with the number of matings, suggesting that female reproduction may regularly be limited by sperm availability (reviewed by Ridley 1988; Arnqvist and Nilsson 2000; see also Simmons 1988; Baker et al. 2001). The females in our study, which were mated twice early in life, likewise appear to have been sperm limited. On average, only 46.8% of the eggs produced by females showed evidence of fertilization, and female fertility declined over time, in part because proportionally fewer eggs showed evidence of fertilization. Because males that produce faster chirps appear to transfer more sperm in their spermatophores, females that were mated to these males may have been able to fertilize a higher proportion of their eggs, thereby increasing their lifetime fertility. Females could also reduce the risk of sperm limitation by mating more frequently. There may be costs, however, of searching for or being in association with males, such as an increased risk of predation (Magnhagen 1991; Jennions and Petrie 1997). Correlations between male traits preferred by females and the quantity of sperm transferred to females during mating have been reported for guppies, *Poecilia reticulata* (Matthews et al. 1997; Pitcher and Evans 2001), although it is not known if mating with preferred males increases female fertility. Fertility benefits of female preferences, however, have been found in animals with external fertilization (Ryan 1980; Robertson 1990; Pfennig 2000).

While our results are consistent with a sperm limitation

mechanism for the female fertility benefit, it is possible that males instead transfer products to females in their seminal fluid that increase the probability that a female's eggs will develop. Because we assessed fertility based on the appearance of landmarks that become visible during early development, we were unable to distinguish between eggs that failed to develop because they were not fertilized and fertilized eggs that failed to develop. In a number of insects, for example, products that males transfer during mating are incorporated into eggs (e.g., Boggs and Gilbert 1979; Markow 1988; Wedell 1993) and these male products may affect egg or offspring survival (Gwynne 1988a,b; Dussourd et al. 1989). In fact, the absence of an effect of male chirp rate on female fertility in the high-nutrition females, despite the low average fertility of these females, suggests that the fertility benefit observed for reduced-nutrition females may not be entirely explained by a correlation between male chirp rate and sperm count. Furthermore, there was a positive effect of male chirp rate on the fecundity of reduced-nutrition females. Males that produce faster chirps may thus transfer products in their seminal fluid that positively affect both female egg production and egg development.

The effect of male phenotype on female reproduction may be due to female processes, such as cryptic choice, rather than male products (Eberhard 1996, 1998). First, females may begin laying eggs more quickly when mated to males that produce faster chirps, and earlier oviposition may result in higher lifetime fecundity. To the extent that lifetime fecundity affects lifetime fertility, earlier oviposition may also increase lifetime fertility. There was no effect, however, of male chirp rate on female age at first reproduction. Second, females may temporarily increase their fecundity, without changing the age at first reproduction, when mated to males that produce faster chirps. This temporary increase in fecundity may then result in higher lifetime fecundity and fertility. There was not, however, an effect of male phenotype on the lifetime fecundity or fertility of females on the high-nutrition diet. It is not clear why females would exercise cryptic choice when provided with a low-quality diet but not when provided with a high-quality diet. In contrast to female fecundity and fertility, it seems unlikely that the effect of male chirp rate on the proportion of eggs fertilized could be a result of cryptic choice. Because eggs fertilized by less-preferred males will have higher average viability than unfertilized eggs, females should not produce a higher proportion of unfertilized eggs when they mate with less-preferred males.

Reduced-nutrition females in our study had an average life span of 54 days after adult eclosion, whereas females in the field are likely to have substantially shorter life spans (Simmons and Zuk 1994; Murray and Cade 1995). It is possible that the fitness differences observed in the laboratory do not occur early enough in life to result in selection on female mating behavior under natural conditions. The effects of male chirp rate on female reproduction are similar, however, when fecundity, fertility and the proportion of eggs fertilized are measured 21 days after adult eclosion, a biologically reasonable life span. Twenty-one days after adult eclosion there was a nonsignificant positive effect of male chirp rate on female fecundity ($t_{23} = 1.54$, $P = 0.138$), a marginally significant positive effect of male chirp rate on female fertility

($t_{23} = 1.93$, $P = 0.066$), and a significant positive effect of male chirp rate on the proportion of a female's eggs that were fertilized ($t_{23} = 2.26$, $P = 0.034$).

Life Span Benefits

Male insects are known to include products in their seminal fluids that have profound effects on female behavior and physiology (Riemann et al. 1967; Baumann 1974; Stanley-Samuels and Loher 1986; Wolfner 2002). Evidence suggests that these products may either reduce or increase female life span; while females in many insects are known to incur longevity costs as a result of mating, there are also many species in which females receive longevity benefits as a result of mating (reviewed by Ridley 1988; Arnqvist and Nilsson 2000). Previous work with *G. lineaticeps* has shown that females that mate more frequently have greater longevity, even when all that they receive from their mates is sperm and seminal fluid (Wagner et al. 2001a). The results presented here further suggest that the seminal fluid of males that produce longer chirps has the greatest positive effect on female life span. Additional work will be necessary, however, to definitively demonstrate that a male seminal fluid product is responsible for the female life-span benefit, and if so, how this product increases life span.

One class of product commonly transferred in the seminal fluid of male insects is material that stimulates rapid oviposition by females (Leopold 1976; Chen 1984; Wolfner 2002). The effect of these oviposition stimulants on female life span is usually not known (but see Lung et al. 2002). Earlier reproduction, however, is known to reduce female life span in a number of animals (Rose 1991; Stearns 1992; Wachter and Finch 1997). A possible explanation for the life span benefit that results from mating with preferred males in *G. lineaticeps* is that males that produce longer chirps transfer fewer or less potent oviposition stimulants to females. In our study, females that were mated to males that produced longer chirps showed a delay in reproduction. This delay in reproduction in turn may have caused an increase in female life span. Whether this effect was due to covariation between chirp duration and the quantity of oviposition stimulants has yet to be tested. If, however, the life-span benefit resulted from the transfer of fewer oviposition stimulants by males that produced longer chirps, this would suggest that females receive qualitatively different benefits from mating more frequently and from mating with males that produce longer chirps; females that mate more frequently would receive relatively more oviposition stimulants. As with the fecundity and fertility benefits of mating with preferred males, it is possible that the effect of male chirp duration on the initiation of reproduction by females was due to female processes rather than due to products transferred by males. It is unclear, however, why females would voluntarily begin laying fertilized eggs sooner when they mate with males that produce a less preferred song type. Instead, females should delay oviposition when they mate with males with less preferred traits because this allows them the opportunity to mate again and have more of their eggs fertilized by more preferred males (Eberhard 1996, 1998).

Another possible cause of the life-span benefit is that males

that produce longer chirps transfer more nutrients in their seminal fluid. Male nutrient donations are common in insects, and include both materials that females consume and materials that are absorbed through the female reproductive tract (reviewed by Vahed 1988; Boggs 1995; Gwynne 1997). In field crickets, the entire spermatophore, including the ampulla and its contents, is very small. Mean spermatophore mass in *G. lineaticeps* is approximately 1.08 mg ($N = 38$, $SE = 0.04$), which represents, on average, 0.22% of a male's mass (W. E. Wagner, unpubl. data). This is consistent with the relative spermatophore masses of other field crickets (e.g., Schaus and Sakaluk 2001), and it is much smaller than the spermatophores of many other insects, which can represent between 1.5–30% of a male's mass (summarized by Boggs 1995). The mass of the seminal fluid is much less than the mass of the spermatophore. Even assuming that the majority of the seminal fluid contains nutrients that females are able to assimilate, it seems unlikely that this small quantity of nutrients would have a meaningful effect on a female's energy budget. It is possible, however, that males transfer trace nutrients in their seminal fluid, and that these trace nutrients positively affect female life fitness (e.g., Gwynne 1988a; Boggs 1990).

Across the two diet treatments, females that lived longer had higher lifetime fecundity, but this did not result in higher lifetime fertility (see the Female Sperm Limitation section of the Results). The absence of an effect of life span on lifetime fertility may have been an artifact of our experimental design. Because females were mated only twice, with both matings occurring within 24 days of adult eclosion, females may have become increasingly sperm limited as they aged, resulting in a decline in fertility. Under natural conditions, females would often have the opportunity to mate again, and a longer life span might therefore allow females to produce more fertile eggs.

Effect of Diet on Female Benefits

Female *G. lineaticeps* on the high-nutrition diet lived longer than females on the reduced-nutrition diet, but there was no difference between the groups in lifetime fertility, lifetime fecundity, or the proportion of eggs fertilized. Dietary restriction of between 40% and 80% is known to increase life span in some animals by 20% to 80%, often with a simultaneous reduction in reproductive output (reviewed by Weindrich and Walford 1988; Yu 1993). It is not known why reduced-nutrition females in our study had shorter life spans and no decline in reproduction. There is variation among species, however, in the effects of dietary restriction. In the Mediterranean fruit fly, *Ceratitis capitata*, dietary restriction causes both a reduction in life span and a reduction in reproduction (Carey et al. 2003). These differences among species in the effects of dietary restriction may reflect differences in life-history strategies. Survival rates of field crickets under natural conditions are likely to be low (Simmon and Zuk 1994; Murray and Cade 1995). Females experiencing a low-quality diet may thus benefit most from maintaining high levels of reproduction at the cost of a reduced life span. It is notable that male *G. lineaticeps* provided with a higher-quality diet appear to use this additional energy to increase

their calling effort instead of storing energy for future use (Wagner and Hoback 1999).

Females on the two diet treatments not only differed in life span, they also differed in the effect of mating with males that produced preferred song types. While females placed on a reduced-nutrition diet directly benefited from their mating preferences, females placed on a high-nutrition diet did not receive these same benefits. This result suggests that male ejaculate products may be most important to females when they are nutritionally or physiologically stressed. When a female's nutritional history affects the benefits of mate choice, this should favor plasticity in female preferences; females should be more discriminating when food is scarce than when food is plentiful. It is not known whether nutritional history affects the mating preferences of female *G. lineaticeps*, but nutritional history is known to affect aspects of female behavior in other animals. For example, in the Mormon cricket, *Anabrus simplex*, males provide nutritional contributions to females during mating, and females compete for access to males. A reduction in diet quality increases the importance of male nutritional contributions, and females fight more for access to males (e.g., Gwynne 1993).

Trade-Offs between Mating Benefits

There was a marginally significant negative correlation between chirp rate and chirp duration in the males that we mated to females in the benefits study. This negative correlation was significant in a field sample of male singing behavior and in a much larger laboratory sample of male singing behavior. Because of the negative phenotypic correlation between chirp rate and chirp duration, females may often have to trade off one type of benefit for another. For example, selecting a male that produces faster chirps and conveys more of a fertility benefit may often result in selecting a male that produces shorter chirps and conveys less of a life-span benefit. Whether females make such trade-offs during mate choice depends on the relative effects of the two benefits on overall female fitness and on how females rank the two male traits. It is possible, for example, that one benefit has a much larger effect on female fitness and that the male trait correlated with this benefit has the largest effect on female mate choice. Very little is known about trade-offs that females make between different mating benefits, but understanding these trade-offs and the conditions under which different benefits are most important is essential for understanding the evolution of female mating preferences.

Reliability of Male Signals

The evolution of reliable signaling of direct benefits is fundamentally different from the evolution of reliable signaling of male genetic quality (e.g., Kokko 1998). One difference is in how signal reliability is maintained. Reliable signaling of both direct and indirect benefits requires mechanisms that prevent deceptive signaling by low-quality males (i.e., the production of attractive signals by males unable to provide the mating benefit). This is thought to occur when a given increase in signal value has a greater marginal fitness cost for males of lower-quality than for males of higher quality (Zahavi 1975; Nur and Hasson 1984; Grafen 1990). Under

such conditions, the fitness of low-quality males is maximized when they produce less attractive mating signals, while the fitness of high-quality males is maximized when they produce more attractive mating signals. Only a small number of studies have explicitly addressed the marginal fitness costs of producing preferred signals (e.g., Møller 1989; Møller and de Lope 1995). Reliable signaling of direct benefits, but not indirect benefits, also requires mechanisms that prevent deceptive signaling by high-quality males. Deceptive signaling by high-quality males is irrelevant when male signals provide information about male genetic quality; a male cannot choose to withhold the benefit from females, and a male is unlikely to ever benefit from doing so. In contrast, a male potentially can produce a signal indicating that it will provide a high-quality direct benefit to females, and while the male is capable of providing such a benefit, it may choose to withhold the benefit once a female has been attracted. Unless females possess mechanisms that punish males that withhold direct benefits, selection should often favor deceptive signaling by high-quality males; such males would obtain the benefit of mating without paying the cost of providing the benefit to females.

Retaliation by females against high-quality males that withhold a mating benefit may be favored when females can directly assess the quality of the benefit prior to mating. When the benefits are received after mating, however, such as would be true for benefits that result from products transferred in male seminal fluid, retaliation by females should not be favored by selection; once a benefit has been received, whether females use a male's sperm to fertilize its eggs should be based on the future costs and benefits of using the male's sperm, not on whether the female was cheated in the past on a direct benefit (e.g., Simmons and Parker 1989). There are a number of reasons, however, that selection may favor the evolution of traits in females that have the consequence of punishing males that transfer lower-quality benefits. For example, selection may favor females that mate fewer times with a male that transfers a lower-quality benefit. This may occur because the benefits of additional matings do not outweigh the costs of remaining in association with the male. Males that transfer lower-quality benefits would consequently transfer fewer sperm to females. In addition, selection may favor females that mate more rapidly with another male when they receive lower-quality benefits from the current male. This may occur because females more rapidly require additional male resources. Males that transfer lower-quality benefits would consequently experience more intense sperm competition. Finally, if there are costs of sperm storage, females may only store a male's sperm if they receive sufficient benefits to compensate them for these costs. Such mechanisms would evolve for reasons independent of punishing deceptive males, but once they have evolved, they would have the consequence of punishing males that provide lower-quality benefits. Furthermore, when females possess mechanisms that punish males that provide lower-quality benefits, males that provide higher-quality benefits should benefit more from a given investment in signaling. Differential benefits of signal investment can maintain reliable signaling (Getty 1998).

The factors that constrain deceptive signaling by males of

lower quality in *G. lineaticeps* are not known. One possibility is that preferred signal types are energetically more expensive to produce, and that only males in good condition can afford to pay the costs of both producing preferred signal types and providing mating benefits to females. Faster chirps are known to be more expensive for male *G. lineaticeps* to produce (Hoback and Wagner 1997), and males produce faster chirps when they are provided with a higher-quality diet (Wagner and Hoback 1999). Male chirp rate thus covaries with nutritional condition. Whether males in better condition also transfer more sperm or fertility-enhancing seminal fluid products to females is not known, although male sperm counts are known to vary with nutritional factors in other animals (e.g., Birkhead et al. 1998). Longer chirps, however, do not appear to be more expensive to produce (Hoback and Wagner 1999), and chirp duration does not appear to be affected by diet quality (Wagner and Hoback 1999). It is also not known why males that produce preferred song types carry through and provide mating benefits to females. One possibility is that males benefit from transferring these products to females. Male-derived products, for example, may affect offspring survival (e.g., Eisner and Meinwold 1987; Gwynne 1988a,b). Under such conditions, males may have evolved to signal the quality of their ejaculates and females may have evolved preferences based on these signals, but the primary determinant of male ejaculate characteristics would be the costs and benefits paternal investment. Alternatively, females may possess mechanisms that punish males that provide lower-quality benefits. If so, female mate choice may affect the evolution of male ejaculate characteristics.

Intersexual Coevolution

Male *G. lineaticeps* appear to transfer beneficial ejaculate products to females during mating (Wagner et al. 2001a), and the results presented here suggest that female calling song preferences favor the transfer of these beneficial ejaculates. A number of recent discussions of the evolution of male and female reproduction have emphasized that conflicts of interest between the sexes can drive sexually antagonistic coevolution (e.g., Stockley 1997; Holland and Rice 1998; Arnqvist and Nilsson 2000). This is particularly apparent in species male traits favored by male-male or sperm competition result in mating costs for females (e.g., Rice 1996; Arnqvist and Rowe 2002). The degree to which the interests of males and females diverge, however, may vary among species. In animals where female choice has a large effect on male reproductive success, males that provide the greatest mating benefits to females should be favored by female mating preferences. Because these males benefit from female mating preferences, the interests of females and preferred males should be more similar than in species in which female choice has less of an effect on male reproductive success. This confluence of interests will be even greater if males that provide beneficial products to females not only have increased mating success, but also if the products provided by males increase other aspects of male fitness. Although there are clearly conflicts of interest between the sexes in most animals, the magnitude of these conflicts and their evolutionary consequences may be variable.

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