Senescence in field crickets (Orthoptera; Gryllidae): examining the effects of sex and a sex-biased parasitoid

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Abstract: The evolutionary theory of aging proposes that senescence is related to decreased selection against deleterious mutations acting late in life. Senescence, i.e., an increase in intrinsic mortality with age, should reflect levels of extrinsic mortality early in life. We tested these predictions using two species of field cricket, *Gryllus integer* and *Gryllus pennsylvanicus*. *Gryllus integer* males are host to a sex-biased parasitoid fly, which orients to the male calling song. As a result, males have reduced life expectancy compared with females in the field. In contrast, *G. pennsylvanicus* males and females appear to have similar life expectancies in the wild. Thus, we predicted that there would be a significant species × sex interaction, with *G. integer* males having the shortest life-span. In two replicates, we found that males of both species died at a significantly younger age than females. However, no evidence of a species × sex interaction was found: in the first replicate, *G. integer* males died earliest, in the second replicate, *G. pennsylvanicus* males died earliest.

Résumé : La théorie évolutive sur le vieillissement suppose que la sénescence est reliée à une sélection moins importante contre les mutations délétères qui agissent au cours de la phase tardive de la vie. La sénescence, c.-à-d. l'augmentation de la mortalité intrinsèque en fonction de l'âge, devrait refléter les degrés de mortalité extrinsèque du début de la vie. Nous avons vérifié ces prédictions chez deux espèces de grillons, *Gryllus integer* et *Gryllus pennsylvanicus*. Les mâles de *G. integer* servent d'hôtes à une mouche parasitoïde qui a une préférence pour les mâles et qui les trouve en s'orientant d'après leurs cris d'appel. Il en résulte que les mâles ont une espérance de vie réduite par rapport à celle des femelles en nature. Par ailleurs, les mâles et les femelles de *G. pennsylvanicus* semblent avoir la même espérance de vie en nature. Nous avons donc posé en hypothèse qu'il y aurait une interaction significative espèce × sexe par laquelle les mâles de *G. integer* doivent avoir la longévité la plus courte. Dans deux expériences répétées, nous avons constaté que les mâles des deux espèces meurent à un âge significativement plus jeune que les femelles. Cependant, nous n'avons observé aucun indice d'interaction espèce × sexe : dans la première expérience, les mâles de *G. integer* sont morts avant alors que, dans la seconde, les mâles de *G. pennsylvanicus* sont morts les premiers.

[Traduit par la Rédaction]

Introduction

The evolutionary theory of aging proposes that an increase in intrinsic mortality with age (senescence) is the outcome of the decreased strength of selection opposing age-specific deleterious mutations acting late in life (Williams 1957; Rose 1991). These deleterious mutations may have either pleiotropic beneficial effects or be neutral with respect to survival and reproduction when young. Senescence is rarely observed in the wild, presumably because extrinsic mortality (i.e., predation, disease, food limitation, etc.) tends to produce a constant mortality rate with few, if any, individuals actually living to the ages when senescent effects are typically observed in the laboratory (Rose 1991; but see McDonald et al. 1996). Extrinsic mortality affects the strength of selection against intrinsic deterioration with age; species with low rates of extrinsic mortality are predicted to show senescence at later ages than species with high rates of extrinsic mortality. A variety of evidence supports this prediction (reviewed in Comfort 1979; Rose 1991).

The field crickets *Gryllus integer* and *Gryllus pennsylvanicus* are well suited to testing predictions derived from the evolutionary theory of aging. In both species, males use specialized forewings to produce a calling song to attract females for mating. Sexual selection theory predicts that males will be the sex to adopt the more risky mate-finding behavior (except in sex-role reversed species; Alexander and Borgia 1979). In *G. integer*, males are attacked by an acoustically orienting parasitoid fly, *Ormia ochracea*, which uses the cricket calling song to locate males (Cade 1975; Gray and Cade 1999). Male crickets infected with fly larvae live about 7 days, dying when the larvae emerge to pupate. *Gryllus integer* females are almost never infected (Cade 1979; A.-M. Murray, unpublished data cited in Murray and Cade 1995). The age structure of *G. integer* in the field suggests that male mortality exceeds female mortality, quite possibly as a direct result of differential parasitoid risk (Murray and Cade 1995). In contrast, *G. pennsylvanicus* is not attacked by a sex-biased parasitoid, and males and females have similar life expectancies in the field (Murray and Cade 1995). Thus, in these species, we predicted that late-

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Fig. 1. Survivorship curves for males and females of *Gryllus integer* and *Gryllus pennsylvanicus* (replicate 1). Solid symbols represent females and open symbols represent males.

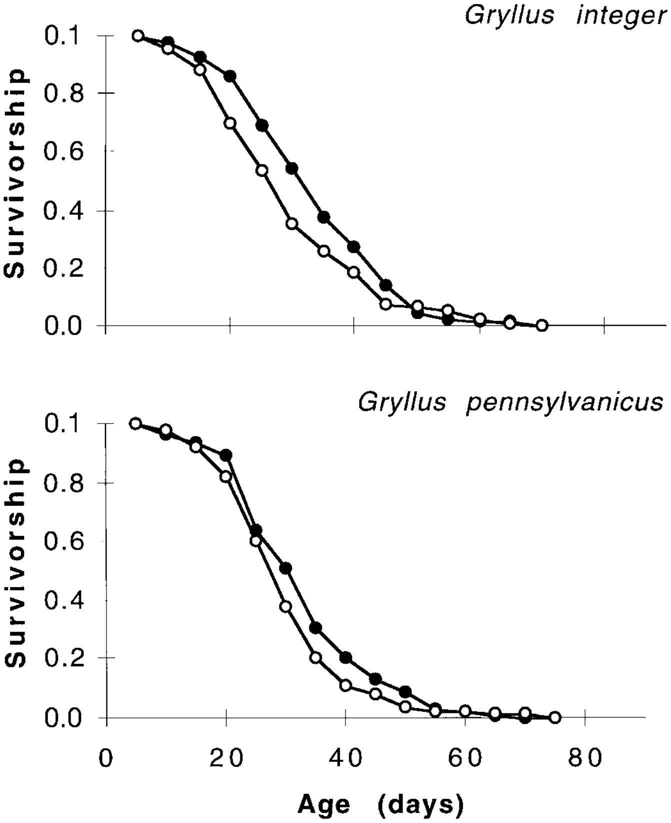
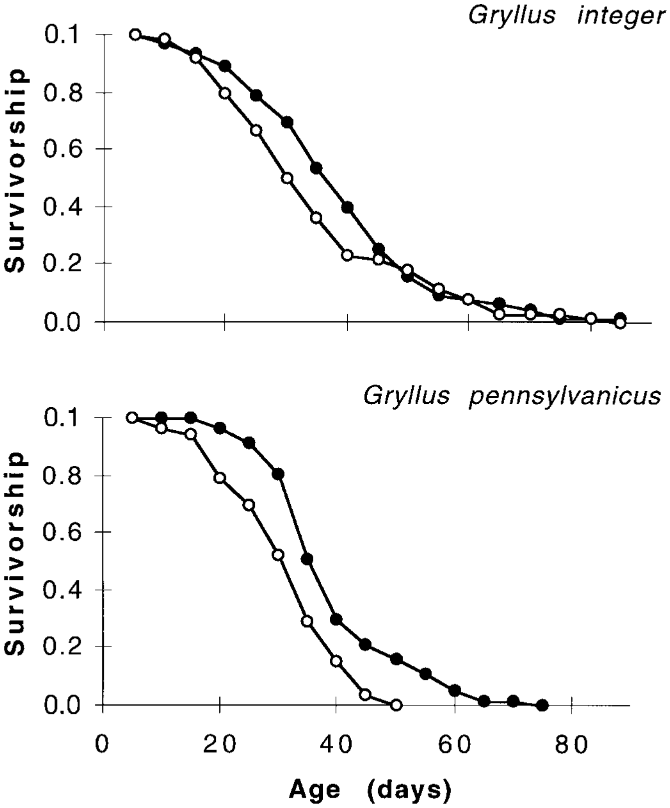


Fig. 2. Survivorship curves for males and females of *G. integer* and *G. pennsylvanicus* (replicate 2). Solid symbols represent females and open symbols represent males.



acting deleterious mutations in the males of *G. integer* are under less intense selection than similar mutations in the females of *G. integer* or in either sex of *G. pennsylvanicus*.

Here we report the results of a study testing this prediction. Specifically, we predicted that *G. integer* males would not live as long as females of either species and that there would be a species × sex interaction, with *G. pennsylvanicus* males having similar laboratory life expectancies as females.

Methods

We measured the longevity of the first generation offspring of field-collected crickets. We used first generation crickets to eliminate the possibility of past selection for enhanced longevity, as an artifact of laboratory conditions, affecting the results. *Gryllus integer* individuals were collected in Austin, Texas, at the Brackenridge Field Laboratory of the University of Texas at Austin and transported to Brock University; *G. pennsylvanicus* individuals were collected in the Niagara region near Brock University. To use only first generation offspring, collections were conducted in both 1997 and 1998. *Gryllus pennsylvanicus* is an obligate egg-diapausing species. Diapause was broken by refrigerating eggs at 4°C for 60 days (Rakshpal 1962). *Gryllus integer* has no obligate diapause. Offspring were reared from eggs at 28°C with a 13 h light : 11 h dark photoperiod. Nymphs were fed cat food and given water ad libitum. Newly eclosed adults were removed from the rearing bins daily. Because body size may affect longevity (Partridge and Farquhar 1983; Nelson and Nolen 1997), we weighed crickets to the nearest 1 mg within 24 h of their adult moult. Mass at the time of moulting is a very good measure of structural body size in crickets (Gray 1997). Crickets were housed individually in containers with ad libitum food and water at the same temperature and photoperiod as the rearing conditions. Containers were checked daily and the longevity of individuals recorded to the nearest 1 day. On six occasions, 1 day was missed. Crickets found dead the following day were given intermediate longevity scores to the 0.5 day ($n = 25$ crickets), so that a cricket found dead 24 days after the adult molt, but following a day that was missed, was given a score of 23.5 days. New adults in the rearing bins were discarded. As senescence can only occur after reproductive maturity (Rose 1991), crickets dying at age 5 days or less ($n = 30$ total) were excluded from the analysis (5 days based on data in Sakaluk 1982; Solymar and Cade 1990*a*). All crickets were unmated and remained so throughout their lives.

Results

Survivorship curves, by species and sex, are given in Figs. 1 and 2. Statistical assumptions that are required for pooling data were not met, thus the data were analyzed separately by replicate. The effect of mass was examined by an analysis of covariance (ANCOVA). The assumption of equal slopes within cells was tested using the method outlined by Barcikowski (1983, p. 318). Slopes were not significantly different, thus justifying this assumption (replicate 1: $F_{[3,352]} = 2.00$, $P > 0.05$; replicate 2: $F_{[3,254]} = 2.37$, $P > 0.05$).

Mass was a significant covariate in replicate 1 but not in replicate 2. To facilitate comparisons, results are presented both from ANCOVA correcting for mass and from an analysis of variance (ANOVA) ignoring mass. There was no effect of species, females lived longer than males, and there was no species × sex interaction (Table 1). Table 2 presents the mean longevities both with and without correcting for mass;

Table 1. Summary of the results of ANCOVA and ANOVA comparing mean longevities of males and females of *Gryllus integer* and *Gryllus pennsylvanicus* for each replicate.

	Replicate 1		Replicate 2	
	$F_{[1,355]}$	P	$F_{[1,257]}$	P
ANCOVA				
Mass	5.44	0.0202	1.23	0.2688
Species	0.75	0.3868	0.22	0.6373
Sex	7.87	0.0053	13.56	0.0006
Species × sex	0.90	0.3446	2.21	0.1387
	Replicate 1		Replicate 2	
	$F_{[1,356]}$	P	$F_{[1,258]}$	P
ANOVA				
Species	0.00	0.9601	0.85	0.3573
Sex	7.81	0.0055	13.11	0.0004
Species × sex	0.46	0.4958	1.83	0.1777

Table 2. Sample sizes and longevities (mean ± SD and least squares mean (LSM)) corrected for mass for male and female *G. integer* and *G. pennsylvanicus* for each of the two replicates.

			Longevity (days)	
Species	Sex	n	Mean ± SD	LSM
Replicate 1				
G. integer	Female	85	32.4±11.6	32.0
	Male	93	28.1±12.7	27.4
G. pennsylvanicus				
	Female	92	31.6±11.9	32.0
	Male	90	29.0±10.7	29.7
Replicate 2				
G. integer	Female	75	37.6±15.4	37.2
	Male	78	33.7±16.0	33.4
G. pennsylvanicus				
	Female	57	38.3±11.2	38.9
	Male	52	29.8±9.5	30.0

clearly the effects of mass were trivial, albeit significant in replicate 1.

Discussion

The mean longevities reported here fall within the range of values reported for a number of cricket species (Masaki and Walker 1987) and a previous study of *G. pennsylvanicus* (Zuk 1987). Male crickets showed reduced longevity compared with females. This finding is consistent with the evolutionary theory of aging (Williams 1957; Alexander and Borgia 1979) and a number of studies of other species (Williams 1957; Rockstein and Lieberman 1958; Comfort 1979; Georgiadis 1985; Rose 1991; Promislow 1992; Promislow et al. 1992). In vertebrates, one of the proximate mechanisms promoting higher male mortality may be pleiotropic effects of testosterone, which enhances reproduction but decreases immune function (Folstad and Karter 1992). Although such effects have not been described in invertebrates, some evidence indicates that there are sex differences in insect immune function (Karp 1985; Rheins and Karp 1985; Gray 1998).

Despite the clear difference between males and females, there was no species × sex interaction. This was not in accord with our predictions. Because of the effects of a sex-biased parasitoid in *G. integer*, we predicted that *G. integer* males would die sooner than *G. integer* females, but *G. pennsylvanicus* males and females would have similar longevities. There was no strong evidence of this in replicate 1 or 2. In replicate 1, *G. integer* males died soonest, whereas in replicate 2, *G. pennsylvanicus* males died soonest. The departure of the data from our predictions was caused by *G. pennsylvanicus*. We predicted that *G. pennsylvanicus* males and females would show similar longevity, thus causing the species × sex interaction. However, this was not the case as *G. pennsylvanicus* males died sooner than females in a manner similar to the sex-biased mortality found in *G. integer.* Thus, the effect of sex seems predominant over any effects of extrinsic mortality.

There are a number of nonmutually exclusive explanations for the absence of the predicted species × sex interaction. We focus here only on the few we consider most likely. Males of both species appear more susceptible to mortality at later ages than females. This is potentially due to males of both species adopting the more risky strategy during their reproductive life-span (Alexander and Borgia 1979). Such an argument suggests that *G. pennsylvanicus* males should in fact die sooner than females when mortality is measured in the field; a prediction not supported by the available data (Murray and Cade 1995). Alternatively, the earlier laboratory senescence of males may be due to the fundamental reproductive difference between males and females. Female crickets fertilize eggs using stored sperm and can continue to produce offspring only while they remain alive. In contrast, female sperm storage allows males to father offspring well after they themselves have died. Female remating and sperm mixing will tend to diminish but may not eliminate this effect. Both female remating and sperm mixing have been described in *G. integer* (Backus and Cade 1986; Solymar and Cade 1990*b*).

Although we failed to find the predicted species × sex interaction, we note that our comparative test used only one species of each type. Further study of other species may provide more support for the hypothesis. Several other species of crickets and katydids are potential candidates: the North American field crickets *Gryllus rubens* and *Gryllus lineaticeps* are also attacked by the same parasitoid fly, as is the Hawaiian population of *Teleogryllus oceanicus* (Walker and Wineriter 1991; Zuk et al. 1995; Wagner 1996). Unfortunately, the utility of these species for further comparative tests is compromised by phylogeny and recency of association. The three *Gryllus* species are each others closest relatives (A. Zera, unpublished molecular phylogeny) and thus do not represent independent tests (Harvey and Pagel 1991); both *T. oceanicus* and *Ormia ochracea* were introduced to Hawaiʹi and so their association may be too recent for an effect to be observed. Perhaps better comparative tests can be made using species of katydids, which also face different risks of attack from acoustically orienting parasitoid flies, e.g., *Poecilimon veluchianus* produces a monosyllabic song and has an average parasitism rate of 14%, whereas its congener, *P. mariannae*, produces a polysyllabic song and has an average parasitism rate of 51% (Lehmann and Heller 1998). Clearly more comparative studies of the life-history effects of acoustically orienting parasitoids is warranted.

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References

Alexander, R.D., and Borgia, G. 1979. On the origin and basis of the male–female phenomenon. *In* Sexual selection and reproductive competition in insects. *Edited by* M.F. Blum and N.A. Blum. Academic Press, New York. pp. 417–440.

Backus, V.L., and Cade, W.H. 1986. Sperm competition in the field cricket *Gryllus integer* (Orthoptera: Gryllidae). Fla. Entomol. **69**: 722–728.

Barcikowski, R.S. (*Editor*). 1983. Computer packages and research design. Vol. 2: SAS. University Press of America, Lanham, Md.

Cade, W.H. 1975. Acoustically orienting parasitoids: fly phonotaxis to cricket song. Science (Washington, D.C.), **190**: 1312–1313.

Cade, W.H. 1979. The evolution of alternative male reproductive strategies in field crickets. *In* Sexual selection and reproductive competition in insects. *Edited by* M.S. Blum and N.A. Blum. Academic Press, New York. pp. 343–380.

Comfort, A. 1979. The biology of senescence. 3rd ed. Elsevier, New York.

Folstad, I., and Karter, A.J. 1992. Parasites, bright males, and the immunocompetence handicap. Am. Nat. **139**: 603–622.

Georgiadis, N. 1985. Growth patterns, sexual dimorphism, and reproduction in African ruminants. Afr. J. Ecol. **23**: 75–87.

Gray, D.A. 1997. Female house crickets, *Acheta domesticus*, prefer the chirps of large males. Anim. Behav. **54**: 1553–1562.

Gray, D.A. 1998. Sex differences in susceptibility of house crickets, *Acheta domesticus*, to experimental infection with *Serratia liquefaciens*. J. Invertebr. Pathol. **71**: 288–289.

Gray, D.A., and Cade, W.H. 1999. Sex, death and genetic variation: natural and sexual selection on cricket song. Proc. R. Soc. Lond. B Biol. Sci. **266**: 707–709.

Harvey, P.H., and Pagel, M.D. 1991. The Comparative method in evolutionary biology. Oxford University Press, Oxford.

Karp, R.D. 1985. Preliminary characterization of the inducible humoral factor in the American cockroach (*Periplaneta americana*). Dev. Comp. Immunol. **9**: 569–575.

Lehmann, G., and Heller, K.-G. 1998. Bushcricket song structure and predation by the acoustically-orienting parasitoid fly *Therobia leonidei* (Diptera: Tachinidae: Ormiini). Behav. Ecol. Sociobiol. **43**: 239–245.

Masaki, S., and Walker, T.J. 1987. Cricket life cycles. Evol. Biol. **21**: 349–423.

McDonald, D.B., Fitzpatrick, J.W., and Woolfenden, G.E. 1996. Actuarial senescence and demographic heterogeneity in the Florida scrub jay. Ecology, **77**: 2373–2381.

Murray, A.-M., and Cade, W.H. 1995. Differences in age structure among field cricket populations (Orthoptera; Gryllidae): possible influence of a sex-biased parasitoid. Can. J. Zool. **73**: 1207–1213.

Nelson, C.M., and Nolen, T.G. 1997. Courtship song, male agonistic encounters, and female mate choice in the house cricket, *Acheta domesticus* (Orthoptera: Gryllidae). J. Insect Behav. **10**: 557–570.

Partridge, L., and Farquhar, M. 1983. Lifetime mating success of male fruitflies (*Drosophila melanogster*) is related to their size. Anim. Behav. **31**: 871–877.

Promislow, D.E.L. 1992. Costs of sexual selection in natural populations of mammals. Proc. R. Soc. Lond. B Biol. Sci. **247**: 203–210.

Promislow, D.E.L., Montgomerie, R., and Martin, T.E. 1992. Mortality costs of sexual dimorphism in birds. Proc. R. Soc. Lond. B Biol. Sci. **250**: 143–150.

Rakshpal, R. 1962. Diapause in the eggs of *Gryllus pennsylvanicus* Burmeister (Orthoptera: Gryllidae). Can. J. Zool. **40**: 179–194.

Rheins, L.A., and Karp, R.D. 1985. Effect of gender on the inducible humoral immune response to honeybee venom in the American cockroach (*Periplaneta americana*). Dev. Comp. Immunol. **9**: 41–49.

Rockstein, M., and Lieberman, H.M. 1958. Survival curves for male and female house-flies (*Musca domestica* L.). Nature (Lond.), **181**: 787–788.

Rose, M.R. 1991. Evolutionary biology of aging. Oxford University Press, New York.

Sakaluk, S.K. 1982. Onset of phonotaxis and age at first mating in female house crickets, *Acheta domesticus* (Orthoptera: Gryllidae). J. N.Y. Entomol. Soc. **XC**: 136–141.

Solymar, B., and Cade, W.H. 1990*a*. Age of first mating in field crickets, *Gryllus integer* (Orthoptera: Gryllidae). Fla. Entomol. **73**: 193–195.

Solymar, B., and Cade, W.H. 1990*b*. Heritable variation for female mating frequency in field crickets, *Gryllus integer.* Behav. Ecol. Sociobiol. **26**: 73–76.

Wagner, W.E., Jr. 1996. Convergent song preferences between female field crickets and acoustically orienting parasitoid flies. Behav. Ecol. **7**: 279–285.

Walker, T.J., and Wineriter, S.A. 1991. Hosts of a phonotactic parasitoid and levels of parasitism (Diptera: Tachinidae: *Ormia ochracea*). Fla. Entomol. **74**: 554–559.

Williams, G.C. 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution, **11**: 398–411.

Zuk, M. 1987. The effects of gregarine parasites on longevity, weight loss, fecundity and developmental time in the field crickets *Gryllus veletis* and *G. pennsylvanicus*. Ecol. Entomol. **12**: 349–354.

Zuk, M., Simmons, L.W., and Rotenberry, J.T. 1995. Acoustically-orienting parasitoids in calling and silent males of the field cricket *Teleogryllus oceanicus*. Ecol. Entomol. **20**: 380–383.