Age-related decrease in male reproductive success and song quality in Drosophila montana

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In the present paper, we have studied the effects of aging on male reproductive success and song quality in Drosophila montana. We analyzed the reproductive success of wild-caught males at their normal breeding age during the mating season and after maintaining the males in laboratory from 1 to 5 months. In line with the mutation accumulation theory of aging, none of the factors affecting the reproductive success of wild-caught males during the mating season were related to male longevity. However, mating activity and progeny production of the males decreased with male age. Interestingly, there was no significant variation in progeny production between males at their normal breeding age, whereas at older age, the variation between males becomes significant. The quality of sexually selected song traits deteriorated with male age in concert with the decrease in male reproductive success. The size of the males did not have an effect on male reproductive success at the normal breeding age, but at older age, larger males were able to maintain the sexually selected carrier frequency of the song at higher level than the smaller males. We conclude that by experimentally extending the reproductive age of the males beyond that which they normally experience in nature, it is possible to expose relationships between reproductive success, sexually selected characters, and body size that are not apparent when analyzed for the males at their normal breeding age. Key words: aging, condition, reproductive success, song quality. [Behav Ecol 19:94–99 (2008)]

Lifelong traits, including the traits affecting male mating success and progeny production, are major components of fitness and therefore the primary targets of natural and sexual selection. The strength of selection on these traits, however, is likely to vary depending on the age of the males (Hamilton 1966; Gavrilo and Gavrilo 2002; Partridge and Gems 2002). There are 2 mutually nonexclusive evolutionary theories of aging: the mutation accumulation theory and the antagonistic pleiotropy theory (see e.g., Charlesworth 2001; Gavrilo and Gavrilo 2002; Partridge and Gems 2002). Both the theories are based on the idea that mutations that have deleterious effects only later in life after the normal breeding age will have a greater chance of persisting and accumulating in the population in the face of natural selection. The antagonistic pleiotropy theory suggests in addition that the late-acting deleterious genes may have beneficial effects early in life and are thus favored by selection and will be actively accumulated in populations despite their deleterious effects late in life.

A few studies have directly addressed the question of whether mutations with only late-acting effects occur, but these experiments have not found evidence to support this proposition (Fletcher et al. 1998, 1999; see also Charlesworth and Partridge 1997 and Partridge and Gems 2002). However, evidence is accumulating on age-dependent expression of genes affecting reproduction. For example, about half of the genes downregulated with age in Drosophila melanogaster are linked to reproduction (Girardot et al. 2006), and female fecundity seems to be determined by different loci at different ages, with no genetic correlation between early- and late-age fecundities (Leips et al. 2006).

In theory, correlation between fecundity and old age survival should be nonexistent or negative due to trade-offs between old age survival and the more important fitness components expressed at early age (Hansen and Price 1995). If old age can be reached only by trading it with other fitness components, this might have a bearing on sexual selection. This is because, as Jones et al. (2000) have pointed out, in such a case the older males may be inferior mates because they may have a genetic tendency to buy longevity with the expense of other fitness components. Indeed, Jones et al. (2000) show that in the lekking sandfly, Lutzomyia longipalpis, choosy females discriminate against older males and gain a fitness benefit from their choice.

In contrast to model of Hansen and Price (1995), Kokko (1998) showed how fecundity and old age survival can also be positively related to each other provided that these are both dependent on the quality/condition of the male. In this case, the long life span of the males could act as an indicator of male genetic quality or good genes (Manning 1985; Kokko 1998). In general, the good genes theory of sexual selection is based on the proposition that male secondary sexual signals are honest indicators of male heritable genetic quality (Zahavi 1975, 1977; Andersson 1994; Kotiaho et al. 2001; Tomkins et al. 2004). Traits may function as honest indicators because they are costly to produce and only males that are in good condition can afford to produce them (see e.g., Andersson 1994; Tomkins et al. 2004).

The chief aim of the present study was to determine how aging affects male reproductive success and the quality of male courtship song among field-collected Drosophila montana flies maintained in laboratory beyond their natural breeding age. First, we examined the reproductive success of overwintered males collected in the wild during their mating season and analyzed whether the components of male reproductive success correlate with male size or longevity. This approach enabled us to analyze the correlations between the fitness components early in life with those associated with survival to old age. The aging theories predict a negative correlation (antagonistic pleiotropy theory) or no correlation (mutant accumulation theory) between the two (Hansen and Price...
1995), whereas the good genes theory of sexual selection suggests that both traits depend positively on the quality of the male thus yielding a positive correlation between the two (Kokko 1998). Second, we traced the effects of aging on male reproductive success and the quality of the sexually selected and nonselected components of male courtship song, paying special attention to whether aging increases variation between males in progeny production.

**METHODS**

**Flies**

Overwintered *D. montana* males were collected in the wild with malt baits in Oulanka, Finland (66°22′N, 29°21′E), during the mating season of the flies in early June. The males were maintained individually in sugar–agar vials in a cold room (4 °C) between the experiments and taken in room temperature at least half an hour before the experiment and song recording sessions. The total number of study males in the first set of experiments (age category 1) was 51 (50 of which produced song during the recording session). At age categories 2–5, the experiments were done with the surviving males, the number of study males decreasing from 51 to 32 by the fifth age category.

Females for the experiments and song recordings were from a laboratory strain mo1 (multifemale line established in 1990, Kemi, Finland, 65°40′ N, 23°35′ E). They were sexed and isolated from males at the age of 1–3 days and maintained in malt vials in groups of 10–20 individuals until they were sexually mature (18–25 days). The female vials were kept in constant light simulating the light conditions during the mating season of the flies in wild, at the temperature of 19 ± 1 °C.

**Reproductive success**

To determine male reproductive success, we tested how many matings each male achieved within a 2-h observation period and how many progeny these matings produced. Each male was placed individually in a malt vial with a virgin sexually mature female. After the male had succeeded to mate with the female, he was transferred into a new vial with a new virgin female. This procedure was repeated until the 2-h observation period was finished. Mated females were left in their individual malt vials where the mating had occurred, to lay eggs for 1 week (females lay eggs after copulation for about 6 days; Aspi 1992). The number of emerging offspring of each female was counted weekly, until all the progeny had emerged. The reproductive success of each male (*n* = 51) was tested first time within 3 days after they had been brought into the laboratory. The procedure was repeated 5 times at 1-month intervals (5 age categories). All the experiments were carried out between 8:00 AM and 11:00 AM.

**Courtship song**

Male courtship song was recorded during 3 days after each set of mating tests. Each male was placed individually in a recording chamber with a virgin *D. montana* female. The chambers consisted of a petri dish (diameter = 55 mm, height = 13 mm) with a nylon net roof and a moistened piece of tissue paper covering the floor. The songs were recorded using a JVC condenser microphone and a Sony TC-FX33 cassette player, at the temperature of 20 ± 1 °C. All the recordings were carried out between 8:00 AM and 11:00 AM.

Male songs were analyzed with the SIGNAL Sound Analysis System (Engineering Design, Berkeley, CA). The song traits were measured from 3 separate pulse trains from the oscillogram (Figure 1) or from the Fourier spectra of the song of each male. To determine the quality of the pulse trains, we counted the number of pulses in a train (PN) and measured the length of the train (PTL). The traits determining the individual sound pulses were analyzed for the fourth sound pulse of the pulse trains. Here we counted the number of sound cycles in a pulse (CN) and measured the length of the sound pulse (PL) and the interpulse interval (IPI), that is, the time from the beginning of the pulse to the beginning of the next pulse. The carrier frequency (FRE) of the songs was measured from the Fourier spectra over the whole pulse train. The means of the song traits were calculated for each male at each age category over the traits measured for the 3 pulse trains.

**Longevity**

When not in the experiments, the males were kept in a cold room (4 °C) in sugar–agar vials throughout the study. Males were changed to fresh vials once a month, and their survival was recorded daily, at least 5 days a week. The longevity of the males (days survived in laboratory) was recorded, and the dead males were preserved in 70% ethanol for size measurements.

**Body size**

We measured thorax length, face width, and head width from all males. As the heads of 2 males were damaged and could not be measured, the sample size of the size measurements is reduced by 2. From the above measures, we derived a new parameter, body size, using principal component analysis. In the rest of the analysis, we used the parameter body size (first principal component) as a measure of the male size.

**Statistical analysis**

The data on male mating activity, progeny production, size, longevity, and song traits were normally distributed (or close to it), and we used Pearson’s correlations to study the relationships between the traits. The effect of male age on his reproductive success (the number of matings and the number of offspring per mating) and song parameters were studied using repeated-measures analysis of variance (ANOVA) followed by appropriate contrast analysis. In order to study the independent effects of male identity, male age, and the ordinal of copulation on the number of offspring per mating, we ran a mixed model nested analysis of covariance in which the male identity was entered as a random factor, the age category as a fixed factor, and the ordinal of mating as a covariate. To determine whether the males show variation in the speed of decline in their offspring production during aging, we analyzed the effect of male identity on the number of offspring per mating separately for each age category. From each of
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Effects of aging on sexually selected and nonselected courtship song components

The songs recorded for the males right after collecting them in the wild (age category 1) had a high PN with short IPIs and pulse trains (PTL). Wild-caught males also had a dense pulse structure with short sound pulses (PL) and a high FRE, a character state preferred by the females (e.g., Ritchie et al. 1998).

The effects of male aging were obvious on the quality of male courtship song in PTL, PL, IPI, and FRE but not in PN or CN (Table 2, Figure 4). Repeated-measures ANOVA for the 32 males producing song in all age categories revealed that FRE...
decreased and PL increased linearly during the life span of the males, whereas PTL and IPI increased quadratically with diminishing returns (Table 2, Figure 4). In *D. montana*, the 2 linearly decreasing song traits (FRE and PL) are targets of sexual selection (Aspi and Hoikkala 1995; Ritchie et al. 1998), and the females have also been found to gain indirect benefit in terms of increased offspring survival when mating with the males producing high-FRE song (Hoikkala et al. 1998).

To study whether the age of the flies could have an effect on the relationship between male body size and song quality, we extracted Pearson’s correlation coefficients between male body size and each of the song traits separately for each age category and then correlated the extracted correlation coefficients with the age category of the flies. The analysis revealed a significant correlation for FRE (Table 3). This suggests that as the males get older, larger males are able to maintain the high song FRE (i.e., vibrate their wings at a high pace) better than smaller males. We finally ran a meta-analysis (Rosenthal 1991; Cooper and Hedges 1994; Kotiaho and Tomkins 2002) across the effect sizes of the 6 song parameters to study whether there was an overall positive trend between male body size—song parameter correlation coefficient and the age category of the fly. We found no significant heterogeneity among the effect sizes ($\chi^2 = 2.81$, degrees of freedom = 5, $P > 0.5$) indicating that all relationships are estimating the same true effect size. The overall effect size was moderately high ($r = 0.70$) and significantly different from zero ($Z = 3.70$, $P < 0.001$). Thus, the relationship between male body size and overall song quality became positive with the age of the flies.

### DISCUSSION

The main aim of our study was to determine how aging affects male reproductive success and song quality in *D. montana*. We found no relationship between the factors affecting male reproductive success and male longevity or body size at the normal breeding age of the males. This finding is in line with the mutation accumulation theory of aging predicting no correlation between the fitness components important early in life and those important in surviving to old age (e.g., Hansen and Price 1995; Gavrilo and Gavrilo 2002; Partridge and Gems 2002). Male aging had clear effects on his primary fitness components, mating success, and progeny production, as well as on the quality of his courtship song, although variation between males in progeny production became significant only at older age. Our age manipulation also revealed an unexpected size dependency of the sexually selected song component: as the males got older, larger males were able to maintain the high song FRE better than smaller males.

*Drosophila montana* flies overwinter in northern Finland as adults, mate in spring when the snow melts, and die within 1 month after the mating period (Aspi et al. 1993). All overwintered males are of about the same age (9–11 months) during the mating season, and thus, the age of the males per se may not be an important factor affecting male mating success. In laboratory, the mean life span (time from eclosion to death) of *D. montana* flies is less than 45 days on cornmeal medium (Durbin and Yoon 1987), but it is possible to lengthen the life span of the flies in laboratory by several months by keeping them on sugar diet and slowing down their metabolic rate in cold conditions (Suvanto et al. 1999). In our study, most of the males survived in laboratory to an age that they would have never reached in the wild, which enabled us to study changes in the reproductive success and song quality of the males at a very old age.

Hansen and Price (1995) have suggested that for a greater lifetime reproductive success, it may be more beneficial for the males to invest in survival to maturity and in traits increasing the mating success than in longevity. In our study, age-related changes in male reproduction could be explained by the accumulation of deleterious mutations at older age and also changes in the physiological condition of the males. As Jones et al. (2000), Prowse and Partridge (1997), and Sgro and Partridge (1999) have pointed out, age-related differences in male fecundity may arise through the damaging effects of reproduction earlier in life and/or to a phenotypic decline in

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**Figure 3**
The relationship between the probability that male identity had an effect on the number of offspring per mating and the age category of the flies. Line fitted to the data is a quadratic regression line. The reference line on x axis is the conventional 0.05 significance level.

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**Table 1**
Mixed model nested analysis of covariance for the number of offspring per mating

<table>
<thead>
<tr>
<th>Source</th>
<th>MS</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>300.66</td>
<td>50</td>
<td>1.03</td>
<td>0.428</td>
</tr>
<tr>
<td>Ordinal of mating (age category [male])</td>
<td>306.31</td>
<td>186</td>
<td>1.36</td>
<td>0.006</td>
</tr>
<tr>
<td>Age category (male)</td>
<td>304.57</td>
<td>185</td>
<td>1.35</td>
<td>0.007</td>
</tr>
<tr>
<td>Error</td>
<td>255.94</td>
<td>437</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Table 2**
Repeated-measures ANOVA and linear or quadratic within subjects contrast for all song parameters separately

<table>
<thead>
<tr>
<th>Song parameter</th>
<th>$F$</th>
<th>$P$</th>
<th>Contrast</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PN</td>
<td>1.36</td>
<td>0.252</td>
<td>Linear</td>
<td>1.12</td>
<td>0.297</td>
</tr>
<tr>
<td>PTL</td>
<td>12.28</td>
<td>&lt;0.001</td>
<td>Quadratic</td>
<td>5.50</td>
<td>0.028</td>
</tr>
<tr>
<td>PL</td>
<td>16.92</td>
<td>&lt;0.001</td>
<td>Linear</td>
<td>32.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CN</td>
<td>1.96</td>
<td>0.382</td>
<td>Linear</td>
<td>0.16</td>
<td>0.688</td>
</tr>
<tr>
<td>IPI</td>
<td>40.30</td>
<td>&lt;0.001</td>
<td>Quadratic</td>
<td>30.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Song FRE</td>
<td>13.47</td>
<td>&lt;0.001</td>
<td>Linear</td>
<td>17.84</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

For all ANOVA, df$_1 = 4$ and df$_2 = 124$; for all contrasts df$_1 = 1$ and df$_2 = 31$. 

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sperm quality or sperm transfer ability with male age. The courtship activity of males has been found to reduce male lifespan in *D. melanogaster* (e.g., Partridge and Farquhar 1981; Cordts and Partridge 1996), the mating itself affecting male longevity also through a reduction in male immune function (McKean and Nunney 2001). Also in several other insect species, males have been found to pay a high price for multiple matings (e.g., Kotiaho and Simmons 2003; Martin and Hosken 2004; Paukku and Kotiaho 2005). Given that the evidence for the cost of mating for males is rapidly accumulating, it may be surprising that we found no relationship between the number of matings and longevity. It should be, however, remembered that we studied this relationship only for the males of the first age category, making no efforts to manipulate the mating rate of the males. As Reznick (1992) has shown, the trade-offs between reproduction and other traits may only be evident if the traits are experimentally manipulated.

Cotton et al. (2004) have emphasized that when testing the condition-dependent model of the good genes theory, one should examine a number of stresses, take in account the effects of body size on the studied traits, and study the effects of male condition both in the traits under strong and under weak sexual selection. Here we have used male aging as a stress factor, the reproductive success of the males decreasing along with male aging. We also have taken the male size into account in correlation analysis and traced changes in male song both in sexually selected and nonselected song traits. As expected, the sexually selected traits PL and FRE (see Aspi and Hoikkala 1995; Ritchie et al. 1998) changed in a less preferred direction as the males aged. In contrast, the song traits that are not associated with sexual selection showed no linear changes during aging. This, and the fact that larger males were able to maintain the high song FRE better than smaller males at the old ages, is consistent with the ideas of condition-dependent expression of sexually selected traits, which suggest that

![Figure 4](https://academic.oup.com/beheco/article-abstract/19/1/94/228100/behavioral-ecology)

**Table 3**

Pearson's correlation coefficients (2-tailed test, *P* values in parenthesis) between the male body size—song parameter correlation coefficient and the age of the fly.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age category of the flies</th>
<th>PN</th>
<th>PTL</th>
<th>PL</th>
<th>CN</th>
<th>IPI</th>
<th>Song FRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.342</td>
<td>0.617</td>
<td>-0.343</td>
<td>0.164</td>
<td>-0.108</td>
<td>0.915</td>
</tr>
<tr>
<td>n = 5 in each test.</td>
<td></td>
<td>(0.573)</td>
<td>(0.268)</td>
<td>(0.572)</td>
<td>(0.792)</td>
<td>(0.862)</td>
<td>(0.029)</td>
</tr>
</tbody>
</table>

n = 5 in each test.
traits under intense directional selection should be more sensitive to changes in condition than traits under less intense selection (e.g., Cotton et al. 2004; Tomkins et al. 2004). There is prior evidence for the role of male size in overwintering survival of D. montana (Aspi and Hoikkala 1995), but until this study, all evidence has suggested that size is not an important determinant of male courtship song characteristics.

The present study shows that it is very difficult to separate the effects of accumulation of deleterious mutations and changes in male physiological condition at older age to test reliably the aging theories. However, it also shows that by experimentally extending the reproductive age of the males beyond that which they normally experience in nature, it is possible to expose relationships between reproductive success, sexually selected characters, and body size that are not apparent when analyzed for the males at their normal breeding age. In future, it will be important to establish the generality of these results by extending this approach to other study populations and systems.

FUNDING

Academy of Finland (to A.H., J.S.K., J.O.L.); Jenny and Antti Wihuri Foundation (to M.S.); Graduate school in Evolutionary Ecology (to M.S.).

REFERENCES