Evolutionary Ecology

The effect of mating on starvation resistance in natural populations of Drosophila melanogaster.

--Manuscript Draft--

Manuscript Number:	EVEC1124R2
Full Title:	The effect of mating on starvation resistance in natural populations of Drosophila melanogaster.
Article Type:	Research Article
Keywords:	mating effects; starvation resistance; wild flies; Drosophila melanogaster.
Corresponding Author:	Julieta Goenaga, Ph.D Universidad de Buenos Aires Ciudad Autónoma de Buenos Aires, Buenos Aires ARGENTINA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Universidad de Buenos Aires
Corresponding Author's Secondary Institution:	
First Author:	Julieta Goenaga, Ph.D
First Author Secondary Information:	
All Authors:	Julieta Goenaga, Ph.D
	Julián Mensch, Ph.D
	Juan José Fanara, Professor
	Esteban Hasson, Professor
All Authors Secondary Information:	
Manuscript Region of Origin:	
Abstract:	In nature, behavioural and physiological process involved in mating may entail different costs and benefits for males and females. However, it has been hypothesized that sexual interactions may have additional costs for Drosophila females like decrease in receptivity to remating and shortening of lifespan. During mating, males transfer seminal fluid proteins to females that exert severe physiological changes that may compromise female´s lifespan and reproductive success. However, under specific stressful environmental conditions that organisms usually face in nature, mating may also confer benefits to females. In the present work, we examine the effect of mating on starvation resistance in wild Drosophila melanogaster. We demonstrate that mated females derived from different geographic locations have the benefit of a greater starvation resistance as compared to virgin females. Even though mating status did not affect mean starvation resistance, we detected a strong genotype-specific effect in males. Beyond the obvious advantage of mating, our study reveals that mating might not be perilous for females, as envisaged by sexual conflict theories, but advantageous for flies exposed to shifts in environmental conditions. Thus, our results highlight the importance of studying other ecologically relevant traits that may contribute to the evolution of male-female interactions.
Response to Reviewers:	We detail the actions taken regarding the comments of the Associate Editor: 1. In this study I think the main effects are equally as interesting as the interaction term and they should be interpreted in combination. In the current revision, I think some minor qualification is all that is required in order to avoid the concerns that readers, such as the previous reviewers, might have (mistakenly or not). Following the editor´s advice we decided to give more emphasis on the interaction between line and mating status all along the revised version of the manuscript.

Powered by Editorial Manager® and Preprint Manager® from Aries Systems Corporation

	2. Line 105: Avoiding the mention of "subtle effects of mating status on males" - the effect is strong and line specific - but over all the lines there was no main effect. Please find the correction in lines 106-108 in the revised version of the manuscript. 3. Line 183: perhaps clarify that "the effect of mating status, over all possible iso-female lines, was significant in females but not in males?" Please find the correction in lines 189 in the revised version of the manuscript. 4. Line 196: perhaps include a sentence along the lines of "Therefore, the impact of mating status appears to be line specific and thus accounts for the lack of an overall effect of mating status in males compared to females". Please find the correction in lines 204-206 in the revised version of the manuscript. 5. Line 233: ? and explains the lack of an overall effect of mating status on male starvation resistance. Please find the correction in lines 236-237 in the revised version of the manuscript. 6. Line 238: thus contributing in part to the increase in variance in the mating treatment for female, right? Please find the correction in lines 242-243 in the revised version of the manuscript. 7. Line 240-242: this is simply not true - at the level of the line mating did have effect - it is just that the overall effect is not significant. You can interpret the main effect, but why completely ignore the interactions. What is true is that unlike for females, it is unclear how variation in the number of mating between males would influence the reaction norms. Please find the correction in lines 243-246 in the revised version of the manuscript. 8. I encourage the authors to try to carefully streamline their discussion. The inclusion of the population differences at the start of the interaction seems out of place compared to the general theme of the paper. The a priori expectations (Line 202-210) are not mentioned in the introduction and seem to be not well supported. A discussion of the population differences is required (as per Reviewers 2 suggestion) - I will leave it to the author's whether they tone down the speculation at the start of the discussion and/or move it to elsewhere in the discussion. I also think that the direction of the discussion is overly focused on females - a large portion of the discussion (Line 267 onwards) is on the transfer of peptides. Some discussion is needed on the difference between males and females and the male line specific effects of mating status (without overly lengthening the discussion). This is mentioned briefly Line 262 - 263 and could be made more prominent as an explanation. Following the editor’s advice we decided to give less emphasis to the among population variation and reduced the length of the corresponding paragraph. The discussion of the possible implication of seminal and sex peptides in the effect of the mating status on starvation resistance was reduced (see lines 268-277) and we enlarged the sentences dealing with the comparison between sexes (lines 284-289) and possible implications of the different types of genotype by mating status observed in males and females. Please find the discussion of the male line specific effects of mating status in lines 290-296 in the revised version of the manuscript. Minor comments: 9. Line 67: this should be increased mating rates must be less advantageous for females, mating per se is essential. Please find the correction in the lines 68 in the revised version of the manuscript. 10. Line 103-105: Perhaps it is worth mentioning specifically that for males the impact of mating was iso-line/genotype specific. Please find the correction in the lines 106-108 in the revised version of the manuscript. 11. Line 165: What was used to analyse the data - JMP, R etc etc. Please find the correction in the lines 168-170 in the revised version of the manuscript. 12. Line 183-188: see more detailed comments above. Please see the correction in the point 3 above.

Powered by Editorial Manager® and Preprint Manager® from Aries Systems Corporation

Manuscript
Click here to download Manuscript: Goenaga.doc
Click here to view linked References

The effect of mating on starvation resistance in natural populations of *Drosophila melanogaster*

Julieta Goenaga*, Julián Mensch; Juan José Fanara and Esteban Hasson

Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

Corresponding author:

*Julieta Goenaga

Departamento de Ecología, Genética y Evolución

Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires

Intendente Güiraldes 2160, Ciudad Universitaria, Pab II

Buenos Aires C1428EGA, Argentina

Tel. 54-11-45763348 (ext 421)

Fax. 54-11-45763354

e-mail: julieta.goenaga@gmail.com

Running title: Effects of mating on starvation resistance in wild females flies.

Keywords: mating effects; starvation resistance; wild flies; *Drosophila melanogaster*.

Total word count: 3968
Word counts for:
Abstract: 199
Introduction: 788
Materials and Methods: 840
Results: 480
Discussion: 1477
Acknowledgements: 82
Number of cited references: 66
Number of tables and figures: 2 and 2

Abstract

In nature, behavioural and physiological process involved in mating may entail different costs and benefits for males and females. However, it has been hypothesized that sexual interactions may have additional costs for *Drosophila* females like decrease in receptivity to remating and shortening of lifespan. During mating, males transfer seminal fluid proteins to females that exert severe physiological changes that may compromise female´s lifespan and reproductive success. However, under specific stressful environmental conditions that organisms usually face in nature, mating may also confer benefits to females. In the present work, we examine the effect of mating on starvation resistance in wild *Drosophila melanogaster*. We demonstrate that mated females derived from different geographic locations have the benefit of a greater starvation resistance as compared to virgin females. Even though mating status did not affect mean starvation resistance, we detected a strong genotype-specific effect in males. Beyond the obvious advantage of mating, our study reveals that mating might not be perilous for females, as envisaged by sexual conflict theories, but advantageous for flies exposed to shifts in environmental conditions. Thus, our results highlight the importance of studying other ecologically relevant traits that may contribute to the evolution of male-female interactions.

Introduction

In sexually reproducing organisms, a sexual conflict may arise whenever males and females have different reproductive interests (Partridge and Hurst 1998; Chapman et al. 2003a; Pischedda and Chippindale 2006). The importance of such sexual conflict is its potential to drive evolutionary change via sexually antagonistic co-evolution or co-evolutionary arms race between sexes (Arnqvist and Rowe 2002; Pischedda and Chippindale 2006). Such a scenario may produce adaptations in one sex that could be eventually harmful in the other, leading to subsequent counter-adaptations in the latter sex to alleviate the costs imposed by such harmful adaptations. This is the main hypothesis of the sexual conflict theory (Holland and Rice 1998; Chapman et al. 2003a). For instance, male seminal fluid proteins (SFPs) transferred to females with sperm during mating are potential targets of such sexual antagonistic selection. SFPs induce physiological and behavioural changes in mated females (Chapman and Partridge 1996; Chapman et al. 2003b; Wolfner 2002; Kubli 2003; Avila et al. 2011), increasing male reproductive success by delaying female re-mating and increasing egg laying rate (Wigby and Chapman 2005; Fricke et al. 2009). However, some of these proteins may have toxic side-consequences in females by reducing lifespan and reproductive success (Chapman et al. 1995; Civetta and Clark 2000; Wigby and Chapman 2005). In addition, females may suffer additional costs resulting from sexual interactions as the increase of predation, the risk of physical damage and parasite/pathogen infections and the decrease in the number of interactions with other males (Partridge and Hurst 1998; Arnqvist and Nilsson 2000; Johnstone and Keller 2000; Cordero and Eberhardt 2005). In this context, it has been hypothesized that increased mating rate may be less advantageous for females than for males. Nevertheless, there is some uncertainty regarding the importance and ubiquity of such conflict given the observation of female 'resistance' behavior, the existence of studies that failed to demonstrate the reproductive costs of mating to females (Cordero and Eberhard 2003) and,

72 the very few examples documenting the phenomenon. In support to this point, it has been
73 shown that females of the Australian sheep blowfly (*Lucilia cuprina*) gain rather than lose
74 when responding to changes in male genitalia (Cordero and Eberhard 2003, 2005). Moreover,
75 changes in genital structures may lead to an increase in the access of male seminal products
76 into female's reproductive tract (Cordero and Eberhard 2003, 2005).
77 In nature, organisms often face stressful environmental conditions, which are defined as
78 environmental factors that reduce fitness (Koehn and Bayne 1989). The most important
79 sources of natural selection are the common environmental stressors, desiccation and
80 starvation. Organisms deal with periods of starvation and desiccation by deploying
81 behavioural and physiological mechanisms that allow them to alleviate the consequences of
82 environmental stress (Hoffmann and Parsons 1991; Randall et al. 1997). Studies performed in
83 insect groups as Lepidoptera, Orthoptera and Coleoptera, showed that females mate with
84 multiple males during periods of starvation and desiccation, probably because males transfer
85 nuptial gifts containing large amounts of water and nutrients that may improve female stress
86 resistance (Boucher and Huignard 1987; Butlin et al. 1987; Ivy et al. 1999; Edvardsson 2006).
87 Also, studies in fruit flies of the genus *Drosophila* inhabiting desertic areas have shown that
88 mated females are more resistant to desiccation that unmated females (Knowles et al. 2004,
89 2005). These physiological responses suggests that male-female interactions may affect
90 ecologically relevant traits and indicates that, in a natural scenario, mating may not be as
91 harmful for females as previously thought (Chapman et al. 1995; Wolfner 1997; Lung et al.
92 2002; Chapman and Davies 2004).
93 Usually, flies living in temperate areas are commonly exposed to seasonal variation in food
94 availability. Consequently, the ability of flies to survive during periods of food shortage is an
95 adult fitness component that acquires special relevance during adverse periods (Izquierdo
96 1991; Mitrovski and Hoffmann 2001; Boulétreau-Merle and Fouillet 2002). Starvation

97 resistance is a complex trait influenced by many genes and the external and sexual
98 environments (Harbison et al. 2004; Hoffmann et al. 2005a; Goenaga et al. 2010). Moreover,
99 complex mechanisms are induced that result in an increase of tolerance to food deprivation
100 (Rion and Kawecki 2007).
101 The goal of the present study is to investigate if mating has an effect on starvation resistance
102 in natural populations of *Drosophila melanogaster* uncovering a wide range of environmental
103 conditions. To address this issue, we measured starvation resistance (SR) in mated and virgin
104 flies derived from three sampling localities in Argentina. We demonstrate that mating has a
105 beneficial effect in females as indicated by a greater SR in mated vs virgins in all natural
106 populations tested. Although differences between mated and virgin males in mean survival
107 under starvation conditions were not significant, the effect of mating status on starvation
108 tolerance was genotype (line) specific in males.
109

110 ## Materials and Methods

111 ### Drosophila *stocks*

112 This work was performed with flies collected in three sampling localities from Argentina:
113 Güemes (24º 41'S, 65º 03'W and 695 m above sea level), Cachi (25º 07'S, 69º 09'W and
114 2280 m asl) and Lavalle (32º 50'S, 68º 28'W and 647 m asl). Wild flies were collected using
115 fermented banana baits and sorted by sex upon arrival to the laboratory. Wild inseminated
116 females were isolated and used to establish isofemale lines by rearing the offspring of
117 individual females in vials. All isofemale lines were maintained at a census size of
118 approximately 100 flies per line for 10 generations on a cornmeal-molasses-agar (lab medium)
119 medium at constant temperature (25ºC ± 1), humidity (60% - 70%) and 12 h light / 12 h dark
120 cycle before the onset of the experiments described below. The number of lines analyzed in
121 each locality varied from 7 to11.

122 ***Starvation resistance assays***

123 We estimated SR as the time elapsed (in hours) from the moment in which flies were exposed
124 to the starvation diet until death. The starvation diet consisted of 5 ml of 1.7% agar in distilled
125 water, which provided moisture but not food to the flies. For each line, 200 sexually mature
126 flies of both sexes were released in egg-collecting chambers. In each chamber, a Petri dish
127 containing an egg-laying medium (2% agar in distilled water and baker's yeast) was used for
128 egg collection. Petri dishes were removed after 12 h and incubated at 25 ºC until egg hatching
129 (approximately 24 h). First-instar larvae were transferred to 10 vials (30 individuals per vial)
130 containing lab medium. Adult flies were recovered from the vials and separated by sex under
131 light CO_2 anesthesia and then transferred to vials containing lab medium before SR assays.
132 Therefore, experimental flies were reared under optimal conditions in order to minimize the
133 influence of environmental variation.
134 SR was measured in sets of flies that differed in mating status, mated and virgin flies. To
135 obtain mated flies, adults were recovered from the vials every day and separated by sex. Then,
136 groups of 15 males and 15 females were released in new vials and separated by sex after 3
137 days and maintained in vials (30 individuals per vial) with fresh food before the SR assay.
138 These conditions consistently resulted in females insemination since we detected the presence
139 of larvae in all vials. Although we cannot assure that all flies mated before the SR assay,
140 preliminary experiments revealed that 99% of pairs of virgin females and males tested mated
141 within 10 minutes (results not shown). In order to obtain virgin flies, newly emerged adults
142 were recovered from the vials every 5 hours, separated by sex and maintained in groups of 30
143 individuals of the same sex in vials with fresh food.
144 The SR assay was performed with group of five 3 to 6-day-old flies. Seven to ten vials were
145 set up for each line and sex. All vials were incubated at constant temperature (25ºC ± 1) and
146 under a 12 h light / 12 h dark cycle. Survival was recorded daily at 8.00 h, 14.00 h and 20.00

147 h until the death of all flies. Scores of SR for each individual fly were used to estimate mean
148 survival time per replicate which was the variable considered in all statistical analysis.

149 ***Statistical analysis***

150 A three-way analysis of variance (ANOVA), considering the line as the experimental unit,
151 was performed to analyze variation in SR, according to the following model:

152 $$y = \mu + P + M + S + P \times M + P \times S + M \times S + P \times M \times S + \varepsilon_i,$$

153 where μ is the overall mean, P is the population effect (with three levels: Güemes, Cachi and
154 Lavalle); M is the mating status effect (with two levels: mated and virgin), S is the sex effect
155 and ε_i is the error term which is given by the among line variance. All factors in the ANOVA
156 were considered as fixed. We performed "*a posteriori*" comparisons using Tukey's method
157 when an interaction term was significant.
158 We also performed two-way mixed ANOVAs for each combination of population and sex
159 using the following model:

160 $$y = \mu + L + M + L \times M + \varepsilon_i$$

161 where μ is the overall mean, M (defined above) and L is the line effect (random, with as many
162 levels as the number of lines analyzed in each population). The error, ε_i, is the among
163 replicate variance term. Vials were considered as experimental units in these ANOVAs. In
164 our experimental design differences among lines may be interpreted as an estimation of the
165 genetic component of phenotypic variance, since lines (genotypes) may be considered as
166 different genotypes (David et al. 2005). Likewise, the line by mating status interaction ($L \times M$)
167 may be construed as an estimate of the genotype by environment interaction (G *by* E), which
168 may be interpreted as genetic variation in phenotypic plasticity. Statistical analyses were
169 performed using GLM procedure implemented in the STATISTICA 8.0 software package
170 (StatSoft Inc. 2007).
171

Results

The general ANOVA revealed that populations differed significantly in their tolerance to starvation (Table 1). *A posteriori* pairwise comparisons showed that the significant population effect may be mainly attributed to differences between flies derived from Güemes (52.2 ± 2 h) and Lavalle (46.1 ± 1.1 h) ($p = 0.015$), whereas differences between Cachi (50.8 ± 1.9 h) and the other two populations were not significant. The ANOVA also revealed a significant mating status by sex interaction suggesting that mating differentially affected SR in males and females (Table 1). Further analysis showed that differences between mated and virgin flies were highly significant in females ($p = 0.0001$) but not in males ($p = 0.3$), that differences between males and females were significant in mated ($p = 0.0001$) but not in virgin flies ($p = 0.37$). Actually, mated females outlived virgins by more than 15 h and mated females were significantly more tolerant to starvation than mated males (Figure 1). The non-significant population by mating status by sex interaction suggests that the effect of mating status on SR was homogeneous across populations in both sexes.

Two-way ANOVAs performed for each combination of population and sex showed that differences among lines were significant in all cases (Table 2), indicating that natural populations harbour substantial genetic variation for SR (see also Goenaga et al. 2010). Also, the effect of mating status, over all possible isofemale lines, was significant in females but not in males, pointing to a sex-specific effect of mating status on SR. Though differences in mean SR between mated and virgin females were significant in Güemes and Cachi, and marginally significant in females derived from Lavalle (Table 2), the trends were consistent across all sampling localities. On the other hand, mean SR did not differ significantly between mated and virgin males across all sampling localities. However, it is important to note that the line by mating status interaction was significant in all populations in both sexes (Table 2).

196 The significance of this interaction may be due either to changes in variance among lines
197 across mated and virgin flies and/or departures of the genetic correlation across mated and
198 virgin flies from unity (see Robertson, 1959, Muir et al. 1992). Further analysis showed that,
199 in females, the line by mating status interaction effect could be mainly accounted for a
200 significantly greater variance among lines in mated than virgins in all sampling localities
201 (Figure 2a, Levene's test $p = 0.0001$). In contrast, differences in variance among lines
202 between mated and virgin males were not significant, suggesting that differences in the
203 ranking order among lines across virgin and mated males may account for the significant line
204 by mating status interaction (Figure 2b, Levene's test $p = 0.87$). Therefore, the impact of
205 mating status appears to be line-specific and, thus accounts for the lack of an overall effect of
206 mating status in males compared to females.
207
208 **Discussion**
209 The present study demonstrates that the populations analyzed are slightly differentiated and
210 harbor substantial amounts of genetic variation in their ability to survive to food shortage; and,
211 more importantly, that mating status (virgin vs mated flies) has a strong and homogeneous
212 sex-specific effect on starvation resistance in all populations studied.
213 Concerning among population variation, flies derived from Güemes that inhabit a more
214 benign environment, typical of lowland and tropical areas were, surprisingly, more tolerant to
215 starvation than flies living in harsher environments like in Cachi and Lavalle. These results do
216 not support our hypothesis that flies living in harsher environments, where face longer periods
217 of food shortage, have greater SR (see also Goenaga et al. 2010). These observations are in
218 agreement with the results of reports that propose that the ability to survive periods of food
219 shortage does not correlate with ecologically relevant variables that co-vary with latitude or
220 altitude or climatic variables (Robinson et al. 2000; Hoffmann et al. 2005a; Goenaga et al.

221 2010). Thus, other authors proposed that variation in SR may likely be a consequence of
222 natural selection acting on genetically correlated traits, among which cold resistance is the
223 main candidate (Hoffmann et al, 2002; Hoffmann et al. 2005b; Kenny et al. 2008; Ayroles et
224 al. 2009).
225 Our study also shows that mating may confer a benefit to females by affecting survival under
226 starving conditions. Mated females exhibited an increased tolerance to starvation in
227 comparison to virgins, whereas mating status affected male starvation tolerance in a different
228 way. These observations were visualized as a significant line by mating status interaction in
229 all populations, which under our experimental design may be interpreted as a genotype by
230 environment interaction (*G by E*) since each line can be considered as a different genotype
231 and the mating status a particular type of environmental condition. Such particular *G by E*
232 interaction suggests that the effect of mating status varied across genotypes in both sexes. In
233 females, mating had a beneficial effect on most genotypes in terms of SR and the *G by E*
234 interaction may be accounted for by an increased variance in mated than in virgins. In contrast,
235 the significant *G by E* seems to be due to changes in the ranking order of genotypes across
236 mated and virgin males, which explains the lack of an overall effect of mating status on male
237 SR.
238 However, before further discussing the implications of our results we need to address a
239 critical question of our experimental design, the lack of direct observations of mating.
240 Additional experiments showed that the great majority of pairs mated within 10 minutes after
241 releasing flies in the vials. On one hand, females in our sample of mated flies probably
242 differed in the number of matings achieved during the confinement with males, contributing
243 in part to the increase in variance in the mating treatment in females. On the other hand, our
244 sample of mated males likely included individuals that mated with 0, 1 or more females due

245 to differential mating success before the SR assay and this might have some influence in the
246 reaction norms of males.
247 Similar effects of mating on SR in females have been reported in highly inbred lines with a
248 long history of laboratory rearing (Canton-S and Oregon-R) (Rush et al. 2007) and lines
249 selected for increased lifespan (Service 1989). Interestingly, such positive effect of mating on
250 SR does not seem to be restricted to *Drosophila*. Indeed, mated females of the redback spider
251 (*Latrodectus hasselti*) outlived unmated females when exposed to periods of food deprivation
252 (Stoltz et al. 2010). The authors hypothesized that their observations may be consequence of
253 different investments in mate attraction and reproduction between virgin and mated female
254 spiders. In contrast to these findings, it has been shown that mating is costly for both females
255 and males in the seed bug *Togo hemipterus*, as virgins survived longer than non-virgins when
256 exposed to starvation conditions (Himuro and Fujisaki 2010).
257 The benefit of mating in females may be related to the transference of nuptial gifts during
258 copulation. In fact, it has been reported that in the desert inhabitant *D. mojavensis* (Pitnick et
259 al. 1997; Markow and O'Grady 2005) and other insects, males transfer nuptial gifts that could
260 be incorporated by females and may help to face periods of food and water shortage
261 (Edvardsson 2006; Gwynne 2008). Nevertheless, there is no evidence of nuptial gifts in *D.*
262 *melanogaster*. Hence, the benefit that mating confers to females may be related to other
263 factors that *D. melanogaster* males transfer with sperm, as male accessory gland proteins
264 (*Acps*), which are known to induce changes in female physiology and behavior (Wolfner 2002;
265 Kubli 2003; Chapman and Davies 2004). Among other female post-mating responses (Ravi
266 Ram and Wolfner 2007), it has been shown that food intake increases after mating in
267 *Drosophila* females (Carvalho et al. 2006) and, actually, these results may provide a
268 physiological explanation for the differences in SR between mated and virgin females. A
269 small seminal peptide, the sex peptide (SP), has been identified as a crucial agent to induce

several post-mating responses (Liu and Kubli 2003; Wigby and Chapman 2005; Domanitskaya et al. 2007; Ravi Ram and Wolfner 2007) as sexual maturation and oogenesis in *Drosophila* adult females (Bownes 1989; Riddiford 1993; Soller et al. 1999) which may provoke a rise in food intake (Carvalho et al. 2006) resulting in the accumulation of fat reserves (Rush et al. 2007). Also, it has been shown that the effects of SP on egg laying, receptivity and lifespan are not only dependent on the female's diet (Fricke et al. 2010) but also that diet regulates whether exposure to SP transferring males is beneficial or costly to females (Fricke et al. 2008). Nevertheless, the increase in lipid content seems to be a common mechanism underlying the increase in SR (Chippindale et al. 1996; Harshman and Schmid 1998; Djawdan et al. 1998; Hoffmann et al. 2005b, Goenaga et al. unpublished). Therefore, the ability of mated females to survive longer than virgins to starvation may be explained by previous replenishment of lipid reserves as a consequence of the increase in food intake induced by proteins transferred by males during mating. Anyway, the mechanism through which mated females increase SR remains to be fully established, and is likely to be complex. In contrast, mating did not increase mean SR in mated males as compared to virgins, but affected the trait in a marked genotype-specific way. Unfortunately, studies addressing the putative mechanisms underlying sex differences in the effects of mating on SR (or other stress tolerance traits) are very limited and, most of them focus mainly on females rather than males (see above). Thus, specific experiments testing the hypothesis that the specific responses of males and females to mating are the result of sex-specific feeding responses are necessary. The genotype-specific effect of mating status on male SR is a novel observation in *Drosophila*. Genotypes varied in the magnitude and/or direction of their responses to mating status, implying that mated males of some isofemale lines exhibited greater SR than virgins and *viceversa* in other lines. The genotype-specific effect of mating status suggests some sort of antagonistic effects dependent of the mating status on male SR. Such environment

295 antagonistic effects are particularly interesting, since this type of interaction may contribute to
296 the maintenance of genetic variation for adaptive traits, like SR, in nature.
297 Since in nature organisms are likely to face long periods of food shortage, it is reasonable to
298 assume that SR has an adaptive significance (Breitmeyer and Markow 1988; Hoffmann and
299 Parsons 1991). In this respect, our study shows that mating has, apart from the obvious
300 reproductive advantage, an additional significance that may compensate the disadvantages
301 resulting from the sexual conflict. Moreover, our results indicate that female-male interactions
302 also affect other ecologically relevant traits, indicating that in a natural scenario of low food
303 availability, mating may not be as harmful as envisaged by sexual conflict theories. This is an
304 essential point that should be taken into consideration as some studies indicate that costs and
305 benefits of mating could change depending on local environmental conditions (Candolin and
306 Heuschele 2008; Fricke et al. 2008; Fricke et al. 2010). Indeed, studies in other *Drosophila*
307 have shown that newly mated females are more resistant to desiccation, another trait related to
308 stress tolerance, than virgins in the desert inhabitants *D. mojavensis* and *D. arizonae*
309 (Knowles et al. 2004). In this case, the benefit of mating was explained by the effect of the
310 large male ejaculate, which actively provides substantial nutrients to females (Pitnick et al.
311 1997).
312 In conclusion, the results reported in this study may have important implications in two
313 directions. On the one hand, cast doubts on the "well" established idea that mating may
314 impair female fitness and, on the other hand, open new avenues of research on the benefits of
315 mating under stressful natural conditions.
316
317 **Acknowledgements**
318 The authors wish to thank J A Endler (Editor in Chief), D Hosken and M Hall (Associate
319 Editors) and two anonymous reviewers for critical suggestions and advice that helped to
320 improve previous versions of this paper. This research was supported by grants from

321 CONICET, ANPCyT and Universidad de Buenos Aires. J.G. was recipient of a fellowship
322 from University of Buenos Aires, J.M. is recipient of a fellowship from CONICET. J.J.F. and
323 E.H. members of Carrera de Investigador Científico of CONICET (Argentina).
324

325 **References**

326 Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness
327 in insect. Anim Behav 60: 145-164.
328 Arnqvist G, Rowe L (2002) Antagonistic coevolutoin between the sexes in a group of insects.
329 Nature 415: 787-789.
330 Avila FW, Sirot LK, LaFlamme BA, Rubinstein CD, Wolfner MF (2011) Insect Seminal
331 Fluid Proteins: Identification and Function. Annu Rev Entomol 56: 21-40.
332 Ayroles JF, Carbone MA, Stone EA, Jordan KW, Lyman RF, Magwire MM, et al.: Systems
333 genetics of complex traits in *Drosophila melanogaster*. Nat Genet 2009, 41:299-307.
334 Breitmeyer CM, Markow TA (1988) Resource availability and population size in cactophilic
335 *Drosophila*. Funct.Ecol 12: 14–21.
336 Boucher, L, Huignard J (1987) Transfer of male secretions from the spermatophore to the
337 female insect *Caryedon serratus* (OI.): analysis of the possible trophic role of these secretions.
338 J Insect Physiol 33: 949-957.
339 Boulétreau-Merle J, Fouillet P (2002) How to overwinter and be a founder: egg-retention
340 phenotypes and mating status in *Drosophila melanogaster*. Evol Ecol 16: 309-332.
341 Bownes M (1989) The roles of juvenile hormone, ecdysone and the ovary in the control of
342 *Drosophila* vitellogenesis. J Insect Physiol 35:409–414.
343 Butlin, RK, Woodhatch CW, Hewitt GM (1987) Male spermatophore investment increases
344 female fecundity in a grasshopper. Evolution 41: 221-225.

345 Candolin U, Heuschele J (2008) Is sexual selection beneficial during adaptation to
346 environmental change? Trends Ecol Evol 23: 446-452.
347 Carvalho GB, Kapahi P, Anderson DJ, Benzer S (2006) Allocrine modulation of feeding
348 behavior by the sex peptide of *Drosophila*. Curr Biol 16: 692-696.
349 Chapman T, Partridge L (1996) Female fitness in *Drosophila melanogaster*: An interaction
350 between the effect of nutrition and of encounter rate with males. Proc R Soc B 263:755-759.
351 Chapman T, Arnqvist G, Bangham J, Rowe L (2003a) Sexual conflict. Trends Ecol Evol 18:
352 41-47.
353 Chapman T, Bangham J, Vinti G, Seifried B, Lung O, Wolfner MF, Smith HK, Partridge L
354 (2003b) The sex peptide of *Drosophila melanogaster*: Female post-mating responses analyzed
355 by using RNA interference. PNAS 100:9923-9928.
356 Chapman T, Liddle LF, Kalb JM, Wolfner MF, Partridge L (1995) Cost of mating in
357 *Drosophila melanogaster* females is mediated by male accessory gland products. Nature
358 373:241-244.
359 Chapman T, Davies SJ (2004) Functions and analysis of the seminal fluid proteins of male
360 *Drosophila melanogaster* fruit flies. Peptides 25:1477-1490.
361 Chippindale AK, Chu TJF, Rose MR (1996) Complex trade-offs and the evolution of
362 starvation resistance in *Drosophila melanogaster*. Evolution 50:753-766.
363 Civetta A, Clark AG (2000) Correlated effects of sperm competition and postmating female
364 mortality. PNAS 97:13162-13165.
365 Cordero C, Eberhard WG (2003) Female choice of sexually antagonistic male adaptations: a
366 critical review of some current research. J Evol Biol 16: 1-6.
367 Cordero C, Eberhard WG (2005) Interactions between sexually antagonistic selection and
368 mate choice in the evolution of female responses to male traits. Evol Ecol 19:111-122.

369 Djawdan M, Chippindale AK, Rose MR, Bradley TJ (1998) Metabolic reserves and evolved
370 stress resistance in *Drosophila melanogaster*. Physiol Zool 71:584-594.
371 Domanitskaya EV, Liu H, Chen S, Kubli E (2007) The hydroxyproline motif of male sex
372 peptide elicits the innate immune response in *Drosophila* females. FEBS Journal 274:5659-
373 5668.
374 Edvardsson M (2006) Female *Callosobruchus maculatus* mate when they are thirsty:
375 resource-rich ejaculates as mating effort in a beetle. Anim Behav 74:183-188.
376 Fricke C, Bretman A, Chapman T (2008) Adult male nutrition and reproductive success in
377 *Drosophila melanogaster*. Evolution 62: 3170-3177.
378 Fricke C, Wigby S, Hobbs R, Chapman T (2009) The benefits of male ejaculate sex peptide
379 transfer in *Drosophila melanogaster*. J Evol Biol 22: 275-286.
380 Fricke C, Bretman A, Chapman T (2010). Female nutritional status determines the magnitude
381 and sign of responses to a male ejaculate signal in *Drosophila melanogaster*. J Evol Biol 23:
382 157-165.
383 Goenaga J, Fanara JJ, Hasson E (2010) A quantitative genetic study of starvation resistance at
384 different geographic scales in natural populations of *Drosophila melanogaster*. Genet Res
385 92:253-259.
386 Gwynne DT (2008) Sexual conflict over nuptial gifts in insect. Annu Rev Entomol 53: 83-101.
387 Harbison ST, Yamamoto AH, Fanara JJ, Norga KK, Mackay TFC (2004) Quantitative trait
388 loci affecting starvation resistance in *Drosophila melanogaster*. Genetics 166: 1807-1823.
389 Harshman LG, Schmid JL (1998) Evolution of starvation resistance in *Drosophila*
390 *melanogaster*: aspects of metabolism and counter-impact selection. Evolution 52: 1679-1685.
391 Himuro C, Fujisaki K (2010) Mating experience weakens starvation tolerance in the seed bug
392 *Togo hemipterus* (Heteroptera: Lygaeidae). Physiol Entomol 35: 128-133.

393 Hoffmann AA, Parsons PA (1991) Evolutionary Genetics and Environmental Stress. Oxford
394 University Press, New York, New York.
395 Hoffmann, AA, Anderson A, Hallas R (2002) Opposing clines for high and low temperature
396 resistance in *Drosophila melanogaster*. Ecol. Lett. 5: 614-618.
397 Hoffmann AA, Shirriffs J, Scott M (2005a) Relative importance of plastic vs genetic factors
398 in adaptive differentiation: geographical variation for stress resistance in *Drosophila*
399 *melanogaster* from eastern Australia. Funct Ecol 19: 222-227.
400 Hoffmann AA, Hallas R, Anderson AR, Telonis-Scott M (2005b) Evidence for a robust sex-
401 specific trade-off between cold resistance and starvation resistance in *Drosophila*
402 *melanogaster*. J Evol Biol 18: 804-810.
403 Holland B, Rice,WR (1998) Chase-away selection: antagonistic seduction vs. resistance.
404 Evolution 52: 1-7.
405 Ivy TM, Johnson JC, Sakaluk SK (1999) Hydration benefits to courtship feeding in crickets.
406 Proc R Soc B 266: 1523-1527.
407 Izquierdo JI (1991) How does *Drosophila melanogaster* overwinter? Entomol Exp Appl 59:
408 51-58.
409 Johnstone RA, Keller L (2000) How males gain by harming their mates: sexual conflict,
410 seminal toxins, and the cost of mating. Am Nat 156: 368-377.
411 Kenny M, Wilton A, Ballard WO (2008) Seasonal trade-off between starvation resistance and
412 cold resistance in temperate wild-caught *Drosophila simulans*. Austr J Entomol 47: 20–23
413 Knowles LL, Hernandez BB, Markow TA (2004) Exploring the consequences of postmating-
414 prezygotic interactions between the sexes. Proc R Soc B 271 (Suppl 5), S357-S359.
415 Knowles LL, Hernandez BB, Markow TA (2005) Nonantagonistic interactions between the
416 sexes revealed by the ecological consequences of reproductive traits. J Evol Biol 18: 156-161.
417 Koehn RK, Bayne BL (1989) Towards a physiological and genetical understanding

418 of the energetics of the stress response. Biol. J. Linn. Soc. 37: 157-171.
419 Kubli E (2003) Sex-peptides: seminal peptides of *the Drosophila* male. Cell Mol Life Sci 60:
420 1689-1704.
421 Liu H, Kubli E (2003) Sex-peptide is the molecular basis of the sperm effect in *Drosophila*
422 *melanogaster*. PNAS 100:9929-9933.
423 Lung O, Tram U, Finnerty CM, Eipper-Mains MA, Kalb JM, Wolfner MF (2002) The
424 *Drosophila melanogaster* seminal fluid protein Acp62F is a protease inhibitor that is toxic
425 upon ectopic expression. Genetics 160:211-224.
426 Markow TA, O'Grady PM (2005) Evolutionary genetics of reproductive behavior in
427 *Drosophila*: connecting the dots. Annu Rev Genet 39:263-291.
428 Mitrovski P, Hoffmann AA (2001) Postponed reproduction as an adaptation to winter
429 conditions in *Drosophila melanogaster*: evidence for clinal variation under semi-natural
430 conditions. Proc R Soc B 268: 2163-2168.
431 Muir WM, Nyquist Y, Xu S. (1992). Alternative partitioning of the genotype by environment
432 interaction. Theor Appl Genet 84: 193-200.
433 Partridge L, Hurst LD (1998) Sex and Conflict. Science 281: 2003-2008.
434 Pischedda A, Chippindale AK (2006). Intralocus sexual conflict diminishes the benefits of
435 sexual selection. PLoS Biology 4:e356.
436 Pitnick S, Spicer GS, Markow TA (1997) Phylogenetic examination of female incorporation
437 of ejaculate in *Drosophila*. Evolution 51:833-845.
438 Randall D, Burggren W, French K (1997). Eckert Animal Physiology: Mechanisms and
439 Adaptations. 4th edn. W.H. Freeman & Company, New York.
440 Ravi Ram K, Wolfner MF (2007) Seminal influences: *Drosophila* Acps and the molecular
441 interplay between males and females during reproduction. Integr Comp Biol 47:427-445.

442 Riddiford LM (1993) Hormones and *Drosophila* development. In: Bate M and Martinez Arias
443 A (eds) The Development of *Drosophila melanogaster*. Cold Spring Harbor Laboratory Press,
444 pp 899-939.
445 Rion S, Kawecki TJ (2007) Evolutionary biology of starvation resistance: what we have
446 learned from *Drosophila*. J Evol Biol 20: 1655-1664.
447 Robertson A. (1959). The sampling variance of the genetic correlation coefficient. Biometric
448 15: 469-485.
449 Robinson SJW, Zwaan B, Partridge L (2000) Starvation resistance and adult body
450 composition in a latitudinal cline of *Drosophila melanogaster*. Evolution 54: 1819-1824.
451 Rush B, Sandver S, Bruer J, Roche R, Wells M, Giebultowicz J (2007) Mating increases
452 starvation resistance and decreases oxidative stress resistance in *Drosophila melanogaster*
453 females. Aging Cell 6:723-726.
454 Service P (1989) The effect of mating status on lifespan, egg laying, and starvation resistance
455 in *Drosophila melanogaster* in relation to selection on longevity. J Insect Physiol 35:447-452.
456 Soller M, Bownes M, Kubli E (1999) Control of oocyte maturation in sexually mature
457 *Drosophila* females. Dev Biol 208:337-351.
458 StatSoft (2007). Methods and Applications, Version 8.0 StatSoft, Tulsa, OK.
459 Stoltz JA, Hanna R, Andrade MCB (2010) Longevity cost of remaining unmated under
460 dietary restriction. Func Ecol 24: 1270-1280.
461 Wigby S, Chapman T (2005) Sex peptide causes mating costs in female *Drosophila*
462 *melanogaster*. Curr Biol 15:316-321.
463 Wolfner MF (1997) Tokens of love: functions and regulation of *Drosophila* male accessory
464 gland products. Insect Biochem Molec Biol 27:179-192.
465 Wolfner MF (2002) The gifts that keep on giving: physiological functions and evolutionary
466 dynamics of male seminal proteins in *Drosophila*. Heredity 88:85-93.
467

Figure 1. Effect of mating status on SR in females and males. Bars represent mean of SR ± SEM in virgin (black bars) and mated (white bars) females and males. $p < 0.05$*; $p < 0.001$**; $p < 0.0001$***.

Figure 2. Variation in SR in virgin and mated flies in females (a) and males (b) among lines derived from 3 sampling localities.

Table 1
Click here to download table: Table 1.doc

Table 1. General ANOVA to analyze the effect of populations, mating status and sex on SR.

Source of variation	*df.*	*F*	*p*
Population	2	5.06	***0.008***
Mating status	1	34.41	***< 0.00001***
Sex	1	35.52	***< 0.00001***
Populations x Mating status	2	0.09	0.91
Populations x Sex	2	0.19	0.82
Mating status x Sex	1	13.70	***0.0003***
Population x Mating status x Sex	2	0.51	0.60
Error	92		

df. degree of freedom

Table 2
Click here to download table: Table 2.doc

Table 2. Partial ANOVAs performed for each combination of population and sex.

	Cachi		Güemes		Lavalle	
FEMALES	***df.***	***F***	***df.***	***F***	***df.***	***F***
Line	7	44.54***	6	11.32***	10	76.61***
Mating status	1	12.99**	1	17.57**	1	4.71*
Line x Mating status	7	28.30***	6	15.46***	10	60.12***
MALES	***df.***	***F***	***df.***	***F***	***df.***	***F***
Line	7	19.13***	6	7.35***	10	40.13***
Mating status	1	0.78	1	0.56	1	1.74
Line x Mating status	7	9.11***	6	24.01***	10	25.28***

df. degree of freedom

* $0.05 < p < 0.10$; ** $p < 0.001$; *** $p < 0.0001$

Figure 1
Click here to download high resolution image

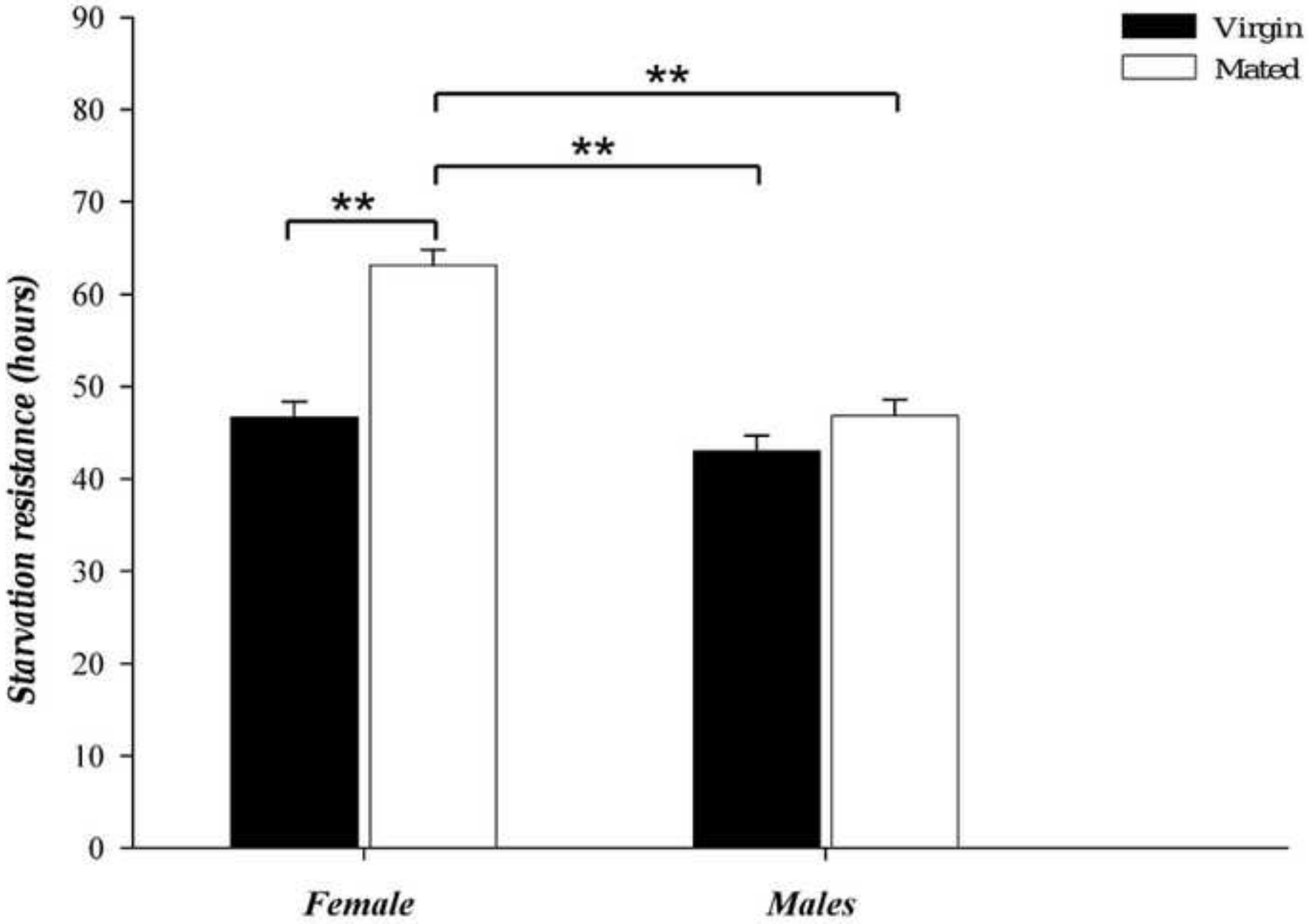
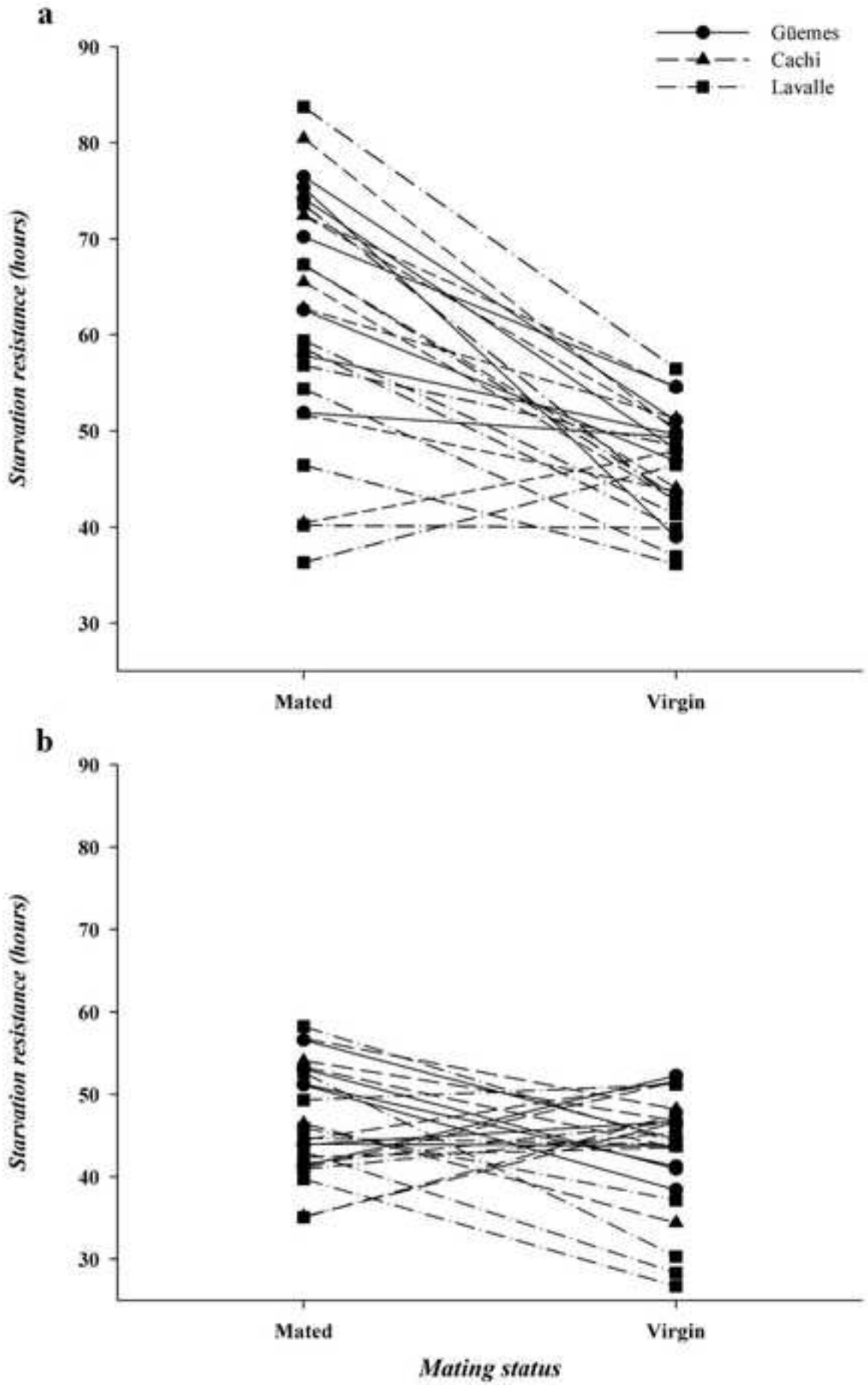
Figure 2
Click here to download high resolution image