



Evolution of life-history traits and mating strategy in males: a case study on two populations of a *Drosophila* parasitoid

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Abiotic and biotic factors affect life-history traits and lead populations to exhibit different behavioural strategies. Due to the direct link between their behaviour and fitness, parasitoid females have often been used to test the theories explaining these differences. In male parasitoids, however, such investigations are vastly understudied, although their mating strategy directly determines their fitness. In this study, we compared the pattern of life history traits and the mating strategy of males in two populations of the *Drosophila* parasitoid *Asobara tabida*, exposed to different biotic and abiotic conditions, with the major difference being that one of them was recently exposed to strong competition with the dominant competitor *Leptopilina boulardi* after recent climate change, the other population being settled in a location where *L. boulardi* has not been recorded. The results showed that individuals of both populations have a different reproductive strategy: in one population, females produced a more female-biased sex ratio, while males accumulated more lipids during their larval development, invested more energy in reproduction and decreased their locomotor activity, suggesting a higher proportion of matings on their emergence patch, all of these factors being possibly linked to the new competition pressure. In both populations, mating without sperm transfer may persist for several days after males become sperm-depleted, and may be more frequent than mating with sperm transfer over their whole lifespan. This point is discussed from an evolutionary point of view. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, 117, 231–240.

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INTRODUCTION

Life-history traits refer to a set of strategies including behavioural, physiological and anatomical traits that impact the fitness of organisms (Ricklefs &

Wikelski, 2002). According to life-history theory, individuals should display a set of life-history traits that maximizes fitness in response to both abiotic (e.g. local climatic conditions) and biotic (e.g. community structure, interspecific interactions such as competition) factors (Roff, 1992; Stearns, 1992, 2000). The evolution of traits is constrained by trade-offs because of limited energy resources (Roff, 1992; Stearns, 1992; Reznick, Nunney & Tessier, 2000;

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Roff & Fairbairn, 2007). As a consequence, environmental conditions not only affect traits in isolation, but can affect the balance of allocation of resources to a large range of life-history traits. As with life-history traits, behaviours, including foraging and reproduction, impact the fitness of organisms, and are subject to energetic constraints. McNamara & Houston (2009) stated that both the internal state of an individual and its life-history traits should be considered to understand how evolution and environment shape these behaviours. Populations or species experiencing different ecological conditions therefore should differ by a set of life-history traits and behavioural traits, making the combined study of both essential to understand how ecological pressures have shaped population features (Réale *et al.* 2010; Niemela *et al.* 2013).

Several factors make parasitoid wasps relevant biological models to study the evolution of life-history traits as well as their consequences in the optimization of behaviours (Godfray, 1994). Parasitoids are arrhenotokous, i.e. a mated female can produce male and female progeny, whereas a virgin female can only produce male progeny. Mated females can control the sex ratio of their offspring, by producing alternatively fertilized and non-fertilized eggs. They frequently produce a highly female-biased due to local mate competition (LMC) (Hamilton, 1967), because the resource (hosts) often has a patchy distribution, and thus a female can optimize her fitness by producing only the number of males required to inseminate her daughters. Females can also adjust their sex ratio according to host size or to different environmental conditions (Charnov, Bull & Mitchell-Olds, 1981). According to the species, the sex ratio can vary from 50:50 to highly female-biased, depending on the host distribution, the insemination capacity of males and male–female conflicts (Hamilton, 1967).

As with females, parasitoid males exhibit complex reproductive behaviours contributing to maximizing fitness. For example, they use chemicals emitted by females as a proxy for patch quality and adjust their residence time according to the density of fertile females (Martel, Damiens & Boivin, 2008; Dufour *et al.*, 2012). Males' reproductive success is generally linked to their ability to inseminate the maximum number of females (Hosken *et al.*, 2009). However, the mating behaviour of males is complex and males can continue to mate even after sperm depletion in many taxa, including insect parasitoids (Damiens & Boivin, 2005; Steiner, Henrich & Ruther, 2008). Such a strategy is viewed as a way to increase their fitness relative to that of other males (Damiens & Boivin, 2006; Steiner *et al.*, 2008). The mating behaviour of males is thus made up of several com-

plementary strategies of fitness optimization. Moreover, recent studies have demonstrated that the internal state of males (e.g. their sperm limitation or feeding status) influences their behaviours (Boivin, Jacob & Damiens, 2005; Martel & Boivin, 2007; Martel *et al.*, 2008; Louapre *et al.*, 2014), highlighting a clear link between reproductive behaviour and physiological states in male parasitoids. The link between life-history traits and mating strategies in males, however, is vastly understudied, in comparison with an extended literature on females.

The guild of larval *Drosophila* parasitoids from the Rhône valley in France represents an interesting system to compare the adaptation of life histories and behaviours in males from different populations. The Rhône valley is a 500-km-long corridor spreading from north to south. The solitary larval endoparasitoid *Asobara tabida* Nees (Hymenoptera; Braconidae) is present in orchards throughout the valley, with *Leptopilina heterotoma* Thompson (Hymenoptera; Figitidae) as native species. A relatively new parasitoid species originating from Africa, *Leptopilina bouhardi* Barbotin (Hymenoptera; Figitidae), has recently expanded its distribution in the south of the valley as a consequence of global warming (Delava *et al.*, 2014) and is now the dominant species (Fleury *et al.*, 2004). *Leptopilina bouhardi*, the presence of which was noted in the south of the Rhône valley for the first time in 1978 (Delava *et al.*, 2014), is, however, still absent in the north of the valley. In the whole valley, hosts of the parasitoid guild include several *Drosophila* species (mainly *D. melanogaster*, *D. simulans*, *D. immigrans*, *D. subobscura* and *D. hydei*). In the southern part of the Rhône valley, *D. melanogaster* and *D. simulans* were the hosts most used by the two *Leptopilina* species. *Asobara tabida*, which cannot parasitize *D. simulans*, mostly used *D. melanogaster*, which is also the favourite host of its new competitor *L. bouhardi* (Fleury *et al.*, 2004). The total parasitism rate was much higher in the south [around 76% in the south (near Valence) compared with around 25% in the north (near Lyon) in 2001]. These observations on community composition and parasitism rates are consistent in the Rhône valley and have been observed since 1988 (Allemand *et al.*, 1999; Fleury *et al.*, 2004). The high rates of parasitism means that intra- and interspecific competition is very high between larval parasitoids (Fleury *et al.*, 2004). To conclude: (1) although the climate varies along the Rhône valley, the studied sites (orchards) are very similar; (2) the host population is also closed, especially for the species used by *A. tabida*; (3) the intensity of competition is higher in the south than in the north; and (4) suitable host availability for *A. tabida* is higher in the north than in the south. Thus, competition pressure is the most

important factor differing between the north and south of the Rhône valley for parasitoids. Competition has increased both recently and rapidly and is very intensive in the south. *Leptopilina boulardi* represents more than 90% of all parasitoid species at the end of September.

Leptopilina boulardi is competitively superior to *A. tabida* when multiparasitism occurs (Fleury *et al.*, 2000); the presence of *L. boulardi* in the south thus results in a higher mortality rate for *A. tabida* larvae (Vayssade *et al.*, 2012; Vuarin *et al.*, 2012), inducing a new competitive pressure for foraging females, affecting life-history traits and behaviours. Previous studies have shown that females from northern and southern populations of the Rhône valley differ in their life-history traits and behaviours (size, metabolic rate, longevity, fecundity, dispersal ability, etc.); females from the southern populations invest more in reproduction (Vayssade *et al.*, 2012; Vuarin *et al.*, 2012), following the predictions of the balanced mortality hypothesis (Price, 1974), which predicts a higher investment in reproduction when competition increases. However, no study has previously been conducted on males from these populations.

The aim of this study was to compare the life-history traits and reproductive behaviours of males from two populations of the parasitoid *A. tabida*, experiencing different ecological pressures, including the interspecific competitor *L. boulardi* (Vayssade *et al.*, 2012; Vuarin *et al.*, 2012). In Rhône valley, the abundance of *A. tabida* is strongly reduced in the south due to competition (Allemand *et al.*, 1999; Fleury *et al.*, 2004), inducing parasitized host patches containing emerging females to be more dispersed. Our main hypothesis was that males from the two populations should differ in their reproductive strategy and life-history traits as they experience different ecological pressures. Males from the southern population should thus allocate more energy to traits linked to reproduction (as observed for females, Vayssade *et al.*, 2012; Vuarin *et al.*, 2012) compared with males from the northern population where host patches are more common. Based on this assumption, we also investigate whether differences in mating strategies align with expected differences in life-history traits, in males from both populations.

MATERIAL AND METHODS

BIOLOGICAL MATERIAL

The *A. tabida* strains used in this study originated from Igé (46.39°N, 4.74°E, altitude 90 m) in the north of the Rhône valley (hereafter 'North strain') and from Avignon (43.95°N, 4.87°E, altitude 100 m)

in the south (hereafter 'South strain'). Both strains were collected using banana-baited traps in 2009. Rearing was started using approximately 50 females and 20 males. The parasitoids were reared on larvae of *D. subobscura* (Diptera: Drosophilidae) (collected in the Netherlands at the end of the 1980s), which are the hosts relevant to the field situation described in the Introduction and the preferred host of *A. tabida*. They were reared in glass jars (Ø: 4.5 cm) containing a substrate of agar–nipagine (the agar serves as a substrate and the nipagine is an antifungal agent) topped with a thin layer of the yeast *Saccharomyces cerevisiae* (Saccharomycetaceae) (produced by Fermipan H); the yeast serves as food for the *Drosophila* larvae. The cultures of both hosts and parasitoids were maintained in a climate room (20 °C, 60% relative humidity, 16:8 h light–dark). The experiments were conducted in 2009 and 2010, approximately 5–10 generations after the individuals were taken from the field.

MALE LIFE-HISTORY TRAITS

A set of life-history traits linked to maintenance, reproduction or dispersal (size, wing load, metabolic rate, locomotor activity, lipid content and longevity, testis volume) were measured for both strains. We estimated the size of the males by measuring the length of their left hind tibia under a microscope (HBO 100 Illuminating System; Zeiss). Wing loading, which constitutes a good estimator of flight capacity (Gilchrist & Huey, 2004), was obtained by calculating the ratio between body mass and wing area. For this, individuals were frozen in liquid nitrogen at emergence, weighed on a microbalance to an accuracy of $\pm 0.1 \mu\text{g}$ (Mettler Toledo XP2U), and photographed under a binocular microscope (0.5 \times 6.3, Olympus SZ-CTV) connected to a video camera (JVC KY-F). The area of the left forewing was then determined using Image J software. Metabolic rate was estimated by flow-through respirometry (Sable Systems International) as the quantity of CO₂ released over 6 h by 1-day-old males (see Vayssade *et al.*, 2012 or Vuarin *et al.*, 2012 for a complete description of the method). Locomotor activity, which is linked to foraging and dispersal, was recorded for 12 h during daylight as the total distance covered during this period by 5-day-old males. Individuals were individually placed in a Petri dish (Ø: 3.7 cm) and followed by a camera (Panasonic CCTV) connected to a computer equipped with Ethovision software (Noldus Information Technology). The individual's position was recorded five times per second using a contrast detection method, under artificial white light at 25 °C. The lipid content of newly emerged males was determined using a colorimetric method

developed by Giron *et al.* (2002), adapted from Van Handel (1985) and as described by Vayssade *et al.* (2012) and Vuarin *et al.* (2012). We measured the males' longevity under starvation (but with access to water) to estimate the amount of energy extracted from the host during larval development. Individuals were isolated into a glass tube, and were monitored daily until death. The investment in reproduction was estimated by measuring the volume of the testis; testis size (either volume or weight) is correlated with reproductive investment (either number of sperm or sperm length) in different groups such as insects, birds and mammals (Møller, 1988, 1989; Pitnick & Markow, 1994; Schradin, Kinahan & Pillay, 2009). In some species, it was possible to count sperm as a measure of reproductive investment, but in these species there were few sperm (< 5000 spermatozoa). In the studied *Asobara* species, spermatozoa numbered more than 20 000. Therefore, it was almost impossible or at least exceedingly costly to count them all. We also cannot use the measure of seminal vesicles due to their vague and diffuse borders. Virgin males of < 1 h were frozen in liquid nitrogen and dissected, and the testes were extracted under a binocular microscope ($\times 4$, Olympus BH2). The testes were photographed and the length (L) and width (w) were measured with the image analysis software ImageJ 1.14o. The volume (V) of the testis was calculated as the volume of prolate spheres with a volume of $V = 1/6\pi Lw^2$. For all the previous traits, the number of repetitions is indicated in Table 1. The sex ratio (i.e. proportion of males) produced by each population was calculated by introducing one randomly mated female (1 day old), which has never oviposited, in a jar with about 200 larvae for 24 h, and by sexing the progeny thus produced. At the end of the experiment, the offspring sex ratio was thus calculated on 272 and 575 individuals produced by six and seven females from the North and South populations, respectively.

MALE MATING CAPACITY AND BEHAVIOUR

The aim of this experiment was to determine males' mating limitation, i.e. how many females a male was able to mate during 6 h, how many of these females were effectively inseminated, how inseminations were distributed during the sequence of mating, and whether these behaviours differ between strains. We used virgin individuals by isolating parasitized pupae from the culture in gelatin capsules (0.68 mL). The experiments were realized in climate rooms, under the same conditions as above for rearing. On the day of their emergence, virgin males were placed individually in a clean

Table 1. Life-history traits of male *A. tabida* strains, from Igé in the north of the Rhône valley, and from Avignon, in the south of the Rhône valley

Life-history trait	Population		Statistics*
	Igé (North)	Avignon (South)	
Mass (mg)	0.37 ± 0.01 (N = 22)	0.30 ± 0.01 (N = 39)	(1) $F = 9.98, P = 0.002$
Tibia length (mm)	0.077 ± 0.001 (N = 23)	0.073 ± 0.001 (N = 36)	(1) $F = 7.18, P = 0.01$
Relative size of testis (mm ³ mm ⁻¹)	0.06231 ± 0.00347 (N = 12)	0.08046 ± 0.01332 (N = 7)	(1) $F = 2.74, P = 0.12$
Wing loading (mg mm ⁻²)	35.33 ± 1.22 (N = 22)	28.48 ± 1.39 (N = 35)	(1) $F = 11.71, P = 0.001$
Metabolic rate (mL CO ₂ h ⁻¹)	1.84 ± 0.23 (N = 20)	3.60 ± 0.68 (N = 22)	(1) $F = 0.83, P = 0.37$
Lipid relative content (µg mL ⁻¹)	0.006 ± 0.0006 (N = 22)	0.009 ± 0.0007 (N = 20)	(1) $F = 9.53, P < 0.004$
Locomotor activity (cm)	2584 ± 386 (N = 8)	1217 ± 114 (N = 18)	(1) $F = 26.59, P < 0.001$
Longevity (days)	10.73 ± 1.64 (N = 168)	14.12 ± 1.87 (N = 193)	(2) β (North) = 0.65, $z = 6.03, P < 0.001$
Sex ratio (proportion of males)	0.39 (N = 272)	0.23 (N = 575)	(3) $\chi^2 = 31.52, P < 0.001$

Data are given as mean ± SE, except for the sex ratio.

*1, one-way ANOVA; 2, Cox proportional hazard model; 3, contingency table.

Petri dish (\emptyset : 8.5 cm) containing a thin agar–nipagine layer and fed with 10% diluted honey for 24 h. The day after their emergence, at 08:00 h, each male was transferred individually to a Petri dish (\emptyset : 4 cm). All females used were virgin, 3–6 days old, fed and kept in pots (\emptyset : 4.5 cm) containing a substrate of agar–nipagine. A first female was introduced and removed as soon as mating ended. Immediately thereafter, another female was introduced; new females were introduced for mating until 14:00 h (i.e. during the period of maximum activity). The female was removed and replaced by another virgin female if she was not mated after 15 min. Typical male mating behaviour in *Drosophila* parasitoids, as for parasitic wasps in general, is as follows: attraction, recognition, orientation, wing vibration (or wing fanning), antennation, head movement, leg tapping, copulation and post-copulatory grooming (Dufour *et al.*, 2012). In this species, females mate only once in their life (Louapre *et al.*, 2014). Each mated female was then placed individually in a rearing glass jar, containing 20–50 larvae of *D. subobscura*. The ability of males to effectively transfer sperm during mating was evaluated by examining the progeny of the mated females: *A. tabida* is arrhenotokous, i.e. a mated female can produce male and female progeny, whereas a virgin female can only produce male progeny. The presence of at least one female in the progeny indicated that sperm was transferred. During the 6 h of the experiment, each mating was recorded using the software The Observer (Noldus, 1995). After the experiment, the tibia length of each tested male was measured as described above to control whether both strains were of similar size. Ten males of the North strain and 14 males of the South strain were used.

SPERM PRODUCTION STRATEGY

Although male parasitoids have mature sperm at emergence (V. Martel, pers. observ.), possible sperm production during their adult life is unknown. To determine if they can produce new sperm after prior reserves have been depleted, 13 sperm-depleted males from each strain from the experiment described above (the last females they mated with produced only males) and aged either 2, 4 or 5 days old were placed with females (one by one, under the same conditions as in the preceding experiment) to evaluate if they were able to produce sperm after depletion. After mating, these females were placed with *Drosophila* larvae, and the sex ratio of the progeny was determined at emergence. As in the previous experiment, if no female emerged, the males were considered unable to produce additional sperm.

LIFESPAN COST OF MATING

The aim of this experiment was to measure the energetic cost (measured as a decrease in longevity) of mating in the two strains. Two days before emergence, parasitized pupae from the culture were isolated in gelatin capsules (0.68 mL) to obtain virgin individuals of known age. Virgin males were assigned to two treatments: in the control treatment, ten 24-h-old males of each strain were placed individually in a clean Petri dish (\emptyset : 8.5 cm) containing a thin agar–nipagine layer (which was replaced every 7 days) and provided with a 10% diluted honey solution *ad libitum* on the day of their emergence. In the second treatment, ten 24-h-old males of each strain were also placed individually in Petri dishes, but were provided with 30 3- to 6-day-old fed virgin females each. The females were removed after 6 h, and the males were kept individually with a 10% diluted honey solution until death. The Petri dishes were monitored daily until all individuals had died. The tibia length (which is correlated with body size) of dead males was measured as described above to control whether both strains were of similar size, as size is known to have an effect on longevity (Vaysade *et al.*, 2012).

STATISTICAL ANALYSES

All statistical analyses were performed using R 2.12.0 Software (R Development Core Team 2010). All means are given \pm SE.

Comparison of male life-history traits

One-way ANOVAs were conducted to compare the life-history traits between the two populations. Sex ratio was analysed with a generalized linear model (GLM; binomial). The effect of the population on the survival rate of males was estimated using the Cox proportional hazard model (Cox, 1972) (package Survival).

Male mating behaviour

First, generalized estimating equations (GEEs, with the clustering term corresponding to the identity of each male tested) followed by an analysis of a ‘Wald statistic’ table were used to test the effects of the rank of the females encountered by the males and the origin of the males on the number of matings with sperm transfer (package geepack). To analyse more specifically the succession of behaviours expressed by the males, the succession of males mating was then considered as the realization of a discrete time Markov chain (DTMC). Given that, a transition matrix was constructed for each strain, to calculate the number of times where the three behavioural items (MWSP, mating without sperm

transfer; M, mating with sperm transfer; R, rejection of the female) followed one another. Comparison between strains was made by the log-likelihood ratio test. In the South strain, some mated females died without laying any offspring, making the precise status of mating (MWSP or M) impossible to assess for these females; we thus performed the test on two extreme situations, one where all undetermined mating statuses were considered fertile (M), and one where all were considered empty (MWSP). We used the more conservative situations by considering the one that gave the least significant results. The transition matrices were represented graphically by an automatic procedure (detailed in Van Baaren & Deleporte, 2001; Van Baaren *et al.*, 2003; Roux *et al.*, 2005), the items being placed on the plan using a correspondence analysis method. Aligned dots on a factorial axis indicate that behavioural events occur in a regular order. Closed dots indicate that a behavioural event follows another one more often than when dots are at a distance (Van Baaren & Deleporte, 2001; Van Baaren *et al.*, 2003; Roux *et al.*, 2005).

RESULTS

MALE LIFE-HISTORY TRAITS

The two strains differed in some life-history traits (Table 1): males from the North strain were significantly heavier and larger, and presented a higher wing load, greater locomotor activity, lower lipid content and reduced longevity without food compared with males of the South strain. Both strains had similar testis volume and metabolic rates. The sex ratio was significantly different between the strains, with a higher proportion of males produced in the North strain.

MALES' MATING CAPACITY AND BEHAVIOUR

In both strains, each male remained active during the whole observation period and displayed courtship behaviours in front of each encountered female. The ten tested males from the North strain successfully inseminated a mean of 13.9 ± 1.2 females per male, mated a mean of 15.2 ± 2.4 females without sperm transfer per male and rejected a mean of 10.5 ± 1.0 females per male during the 6-h period. The 14 tested males of the South strain successfully inseminated a mean of 13.1 ± 1.0 females per male, mated a mean of 18.2 ± 2.9 females without sperm transfer per male and rejected a mean of 11.2 ± 0.6 females per male during the 6-h period. For both strains, the rate of females inseminated increased, but increasingly slowly with the rank of the encountered female (GEE, $\beta = 0.286$, $\chi^2 = 90.4$, d.f. = 1, $P < 0.001$) (Fig. 1). Males

of the North strain successfully inseminated females for a longer period than South strain males during a sequence (GEE, $\beta = 1.882$, $\chi^2 = 4.3$, d.f. = 1, $P = 0.037$); the curve describing the cumulative numbers of females mated with sperm transfer by males of the South strain converge more rapidly than for males of the North strain. Males of the two strains presented a different mating pattern, i.e. a succession of successful inseminations followed by a period where the females were either rejected, or mated without sperm transfer (Fig. 2). This was confirmed by the flow diagrams, which showed that in the North strain (Fig. 2A) there were as many transitions between successful insemination (M) and either rejection of the female (R) or a mating without sperm transfer (MWSP), whereas in the South strain (Fig. 2B), males completed most of their mating with sperm transfer at the beginning of their sequence, and mating without sperm transfer replaced successful insemination only when the male was depleted. The transition matrices are significantly different between both strains, even when all mating was considered successful (M) ($\chi^2 = 16.733$, d.f. = 2, $P = 0.010$). Essentially, the succession of rejections after a mating, fertile or not, is higher in the North strain than in the South strain. The comparison of Figure 2A and B shows that the succession in the South strain is more linear, and governed by a strong succession between mating without sperm transfer (MWSP) and rejections (R) at the end of the sequence.

SPERM PRODUCTION STRATEGY

The results confirmed that depleted males did not transfer sperm because mated females produced

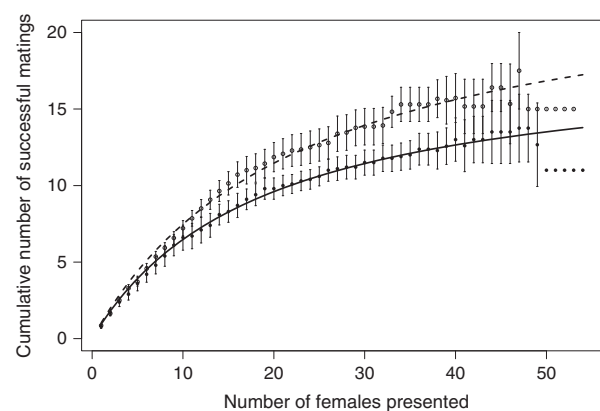


Figure 1. Mean (\pm SE) number of matings with insemination for males of the North strain (open circles) and for males of the South strain (filled circles), depending on the rank of encounter with the females during a mating sequence (unlimited number of females presented one by one to the male, over a period of 6 h).

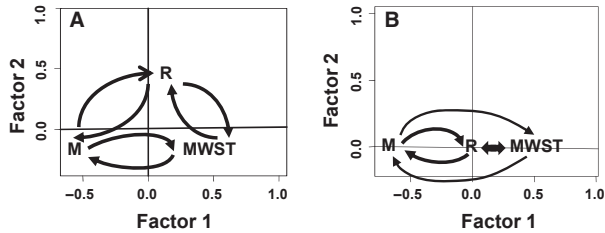


Figure 2. Flow chart on factorial map obtained based on the behaviour of ten males of the North strain and 14 males of the South strain presented with an unlimited number of females one by one over a period of 6 h. Axes I and II are the first two axes of the factorial correspondence analysis. A, North strain; B, South strain. M, mating with sperm transfer; MWST, mating without sperm transfer; R, female rejection. Arrows are representative of the frequency of behavioural successions between two items.

male-only progeny. This was independent of the delay since last mating, indicating that this species is pro-spermatogenic (i.e. males emerge with all their sperm stock and do not produce more during their adult life), or at least have a high spermatogeny index, according to Boivin *et al.* (2005).

LIFESPAN COST OF MATING

Size had no effect on longevity – $z = 1.04$, $P = 0.300$, $\beta = 3.332$, $\exp(\beta) = 27.982$, $SE(\beta) = 3.201$, $z = 1.04$, $P = 0.3$. The interaction between strains and mating was significant, showing that the effect of mating was different for the two strains – $z = -2.35$, $P = 0.019$, $\beta = -1.638$, $\exp(\beta) = 0.194$, $SE(\beta) = 0.698$ (Fig. 3): longevity of males did not differ between virgin and mated individuals from the North strain (respectively, 11.10 ± 1.99 and 11.60 ± 1.70 days) while longevity of virgin males was higher than for mated males from the South strain (respectively 13.22 ± 1.15 and 9.70 ± 1.15 days).

DISCUSSION

Our results confirm that, as for females, males from populations experiencing different ecological conditions differ in their life-history and behavioural traits. Males of the two populations invest equally in sperm production as we found no difference in testis volume (although this may not be the only explanation, it is the more parsimonious). By contrast, mating was found to be more costly in terms of lifespan for males of the South strain than for those of the North strain. However, we do not know whether this cost arises from the quantity of seminal fluids

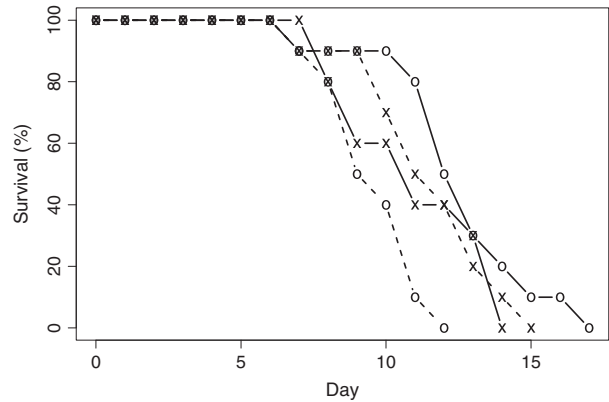


Figure 3. Survival curves of virgin males (continuous line) and males mated with an unlimited number of females over a period of 6 h (dashed line) (open circles, South strain; crosses, North strain).

transferred to the female, from the energy invested in each courtship and copulation event, or from any other physiological expense linked to mating, such as mate location.

Several ecological factors contrasting between the north and the south may explain our results, among them competition pressure, climatic conditions and host distribution. Both populations originate from orchards, meaning that the landscape composition, and fruit distribution and abundance are quite similar between the two. Consequently, the abundance and distribution of *Drosophila* do not vary between the two sites. There is a small difference in mean temperature (Delava *et al.*, 2014), but no site presents stressful temperatures for the native species of *Drosophila* and parasitoids. Competition is thus probably the strongest difference between the two sites, as the difference in competition pressure between both populations is extreme: the total parasitism rate was much higher in the south [around 76% in the south (near Valence) compared with around 25% in the north (near Lyon) in 2001]. These observations on community composition and parasitism rates are consistent in the Rhône valley and have been observed since 1988 (Allemand *et al.*, 1999; Fleury *et al.*, 2004). In the South, at the end of the breeding season, the new competitor represents more than 90% of all species of parasitoids. It is likely that such an increase in competition will affect the traits and behaviours more than a small increase in mean temperature. For females of this species (Vayssade *et al.*, 2012), we observed an evolution of life-history traits that is predicted by theory for an increase of competition. Our observations of the differences in male traits and behaviour are agreement with the observations made on females.

More costly reproduction in the South strain suggests that this energy is invested at the expense of other fitness-related traits according to life-history theory. This result supports (at least for the South strain) the accepted idea that reproduction is also costly for males (Wedell, Gage & Parker, 2002), and males are thus also subject to allocation trade-offs (Lewis, Brakefield & Wedell, 2010). The commonest trade-off found in male insects is between reproductive capacity and the immune system (Drayton, Kobus Boeke & Jennions, 2013). Other trade-offs, between reproduction and dispersal or reproduction and longevity, were found: Gage & Cook (1994) showed that nutritional stress during development may limit sperm production in *Plodia interpunctella* (Lepidoptera: Pyralidae) and Lewis & Wedell (2007) showed that male longevity is negatively correlated with non-fertile sperm production in the butterfly *Bicyclus anynana* (Lepidoptera: Nymphalidae). In *A. tabida*, males of the South strain showed a trade-off between reproduction (through numerous copulations) and longevity and activity (our results showed that they exhibited reduced locomotor activity compared with the North strain).

Males' mating behaviour also differs between the strains. The differences appear in the sequence of successive matings: males of the North strain performed mating without sperm transfer earlier in the sequence, even when subsequent inseminations were successful, whereas males of the South strain began this behaviour later, mostly when they were sperm-depleted. In both strains, as mated females do not mate again (Louapre *et al.*, 2014), the behaviour of mating without sperm transfer gives the male an advantage over conspecifics, reducing the number of receptive females in the population (Steiner *et al.*, 2008).

The observed difference in the distribution of mating with or without sperm transfer between the two strains could be linked to the difference between strains in the frequency of encounters on the natal patch. *Drosophila* parasitoids are protandrous, i.e. males emerge before females (Carton *et al.*, 1986). Soon after emergence, mating is expected to occur mainly locally, leading to frequent inbreeding (Dufour *et al.*, 2012), as predicted by LMC theory (Hamilton, 1967). The sex ratio of the South strain is more female-biased, suggesting a higher encounter rate on the emergence patch, as males emerged before females (Carton *et al.*, 1986). In addition, interspecific competition pressure in the South strain should decrease the number of available patches (Allemand *et al.*, 1999), thus reducing the likelihood and importance of encounters with off-patch females. The best strategy for these males (which are also less active) should thus be to stay on their natal patch

and invest their sperm stock by inseminating a maximum number of females (first their sisters), and stop transferring sperm only when constrained, i.e. sperm-depleted. This strategy, however, implies that matings are done within the progeny of the same female (strategy: all the eggs in the same basket) and is probably not optimal in a situation with lower risk of not finding another patch containing females, as in the North strain.

In both strains males are fully or nearly pro-spermatogenic and can transfer all their sperm in < 1 day providing they encounter enough females. In addition, sperm-depleted males continue to mate with nearly all encountered females, even 4 days after being empty. If males of both strains are able to mate about 30 times in 1 day, to successfully inseminate less than half of them, and to live between 5 and 10 days, non-fertile mating could occur at a very high frequency. Louapre *et al.* (2014) showed that female *A. tabida* mated by a sperm-depleted male behave as virgins, although males do not accept them and prefer to mate with real virgin females. Sexual conflicts are thus particularly notable in this species, and the fact that males are able to perform at least five times more unsuccessful inseminations than successful ones during their lifespan highlights a potential role of this behavior, as a war of attrition or arms race. These results were obtained under controlled conditions while in nature females could possibly escape the sperm-depleted males. Louapre *et al.* (2014), however, showed that females did not reject sperm-depleted males before mating. This behaviour is probably similar to the production of non-fertile sperm at a high rate by male butterflies (Lewis *et al.*, 2010), and would be involved in male competition. Moreover, in other parasitoid species, such as *Nasonia vitripennis* (Chirault *et al.*, 2015) or *Dinarmus basalis* (Lacoume, Bressac & Chevrier, 2007), it has been shown that environmental stresses such as heat or cold produced sperm-depleted males, which females do not reject. This problem of sperm-depleted males could thus have several origins, but if females do not recognize them it will be worth carrying out further research in this area.

In this study, we have shown that males from both strains invest their energy in different life-history and behavioural traits. Vuarin *et al.* (2012) showed that females from these same strains also differ in several traits. Females of the South strain invest more energy in reproduction but have lower dispersal ability and lower longevity with access to food and hosts than females of the North strain. Although all organisms balance the cost of reproduction against other traits, this balance can work differently for males than for females, creating sex-specific

differences in life-history patterns that, in turn, are related to intra- or interspecific competition, or sexual conflicts. Indeed, in this study, the behaviour of sperm-depleted males is obviously deleterious for females, as females mated with a sperm-depleted male will not be accepted by males, although these females behaved as virgins (Louapre *et al.*, 2014).

This study agrees with previous results on females from different populations of the same species (Vaysade *et al.*, 2012; Vuarin *et al.*, 2012), showing variability in patterns of life-history between populations exposed to different abiotic (climate) and biotic (interspecific competition level) factors. In conclusion, males were for a long time vastly understudied in the field of behavioural ecology of parasitoids. This is understandable, given the huge effect of female behaviour and physiology on the fitness of these organisms. However, in this haplo-diploid system, females rely on actual fertilization to produce other females, while males need to maximize the spread of their genes among the former. This is a source of variation in mating behaviour tuning and a possible source of conflict of interest between the two sexes. The case of *A. tabida* gives some insight into this question.

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