Experimental evolution reveals differential evolutionary trajectories in male and female activity levels in response to sexual selection and metapopulation structure

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Behavior is central to interactions with the environment and thus has significant consequences for individual fitness. Sexual selection and demographic processes have been shown to independently shape behavioral evolution. Although some studies have tested the simultaneous effects of these forces, no studies have investigated their interplay in behavioral evolution. We applied experimental evolution in the seed beetle *Callosobruchus maculatus* to investigate, for the first time, the interactive effects of sexual selection intensity (high [polygamy] vs. minimal [enforced monogamy]) and metapopulation structure (yes/no) on the evolution of movement activity, a crucial behavior involved in multiples functions (e.g., dispersal, predator avoidance, or resource acquisition) and thus, closely related to fitness. We found that the interactive effects of the selection regimes did not affect individual activity, which was assayed under two different environments (absence vs. presence of conspecific cues from both sexes). However, contrasting selection regimes led to sex- and context-dependent divergence in activity. The relaxation of sexual selection favored an increase in female, but not male, movement activity that was consistent between environmental contexts. In contrast, selection associated with the presence/absence of metapopulation structure led to context-dependent responses only in male activity. In environments containing cues from conspecifics, males from selection lines under population subdivision showed increased levels of activity compared to those assayed in an environment devoid of conspecifics cues, whereas the opposite was true for males from panmictic lines. These results underscore that both the effects of sexual selection and population spatial structure may be crucial in shaping sex-specific behavioral evolution.

KEY WORDS: Behavior, *Callosobruchus maculatus*, experimental evolution, locomotor activity, metapopulation structure, sex-specific selection.

Behavior constitutes the main mechanism by which animals interact with other individuals, either conspecifics or heterospecifics, as well as with the rapidly and unpredictably changing abiotic environment (Caro 1998; Kappeler 2010).

Behavioral variation thus has significant consequences for fitness of individuals through repercussions in growth, reproduction, or survival. Behavioral variability may also have important ecological and demographic implications by determining the

patterns of mating, dispersion, or migration (Caro 1998; Anthony and Blumstein 2000; Kappeler 2010; Cordero-Rivera 2017). Understanding the causes and consequences of behavioral variation at different hierarchical levels has thus become a central question in evolutionary biology (Caro 1998; Dukas 2006; Réale et al. 2007; Husak and Fox 2008; Kappeler 2010; Careau and Garland 2012; Garamszegi and Møller 2017).

Sexual selection is considered a major force driving the evolution of behavior (Andersson 1994; Arnqvist and Rowe 2005; Kappeler 2010; Schuett et al. 2010). Behavioral traits may be subject to sexual selection when they provide advantage in male-male competition and/or mate choice (Andersson 1994; Kappeler 2010; Schuett et al. 2010; Shuker and Simmons 2014; van Lieshout et al. 2014). Accordingly, there is increasing evidence that behavioral traits act as male quality indicators, signaling direct (e.g., parental ability) or indirect (e.g., increased offspring fitness) benefits for females, thus mediating mate choice (Husak and Fox 2008; Kappeler 2010; Schuett et al. 2010). Further, by imposing different, and sometimes contrasting, selection pressures on males and females, sexual selection may lead to sex differences in the expression of a range of behaviors (e.g., aggression, risk taking, or cooperation), which together may lead to negative genetic correlations for fitness (Poissant et al. 2010; Schuett et al. 2010; Han and Dingemanse 2017; Tarka et al. 2018; Kralj-Fišer et al. 2019). Sexual selection may also induce variation in behavioral traits through life-history trade-offs. For instance, intense male-male competition may favor the evolution of energetically demanding adaptations to enhance male access to females (e.g., more elaborate armaments), at the expense of reducing the expression of traits, including behavioral traits (e.g., for the exploration of the physical and/or social environment) related to other fitness components (Kotiaho 2001; Bond200riansky et al. 2008; Simmons et al. 2017).

Ecological and demographic processes are also key in shaping selection on behavioral traits. The existence of spatial structure may, for example, limit interactions among individuals, altering the reproductive as well as the intraspecific and/or interspecific (e.g., predator-prey, host-pathogens) dynamics of the population, which, in turn, can impact the frequency and expression of behaviors directly affected by these dynamics (e.g., risk taking, exploration, or aggressiveness; Levin 2000; Berger-Tal and Saltz 2019; Lichtenstein et al. 2019). Population subdivision may also have a direct impact on key behavioral modulators of sexual selection such as female mating strategies (Yasui and Garcia-Gonzalez 2016). Further, alterations in the spatial structure of the population (e.g., through habitat fragmentation) often affect the levels of inbreeding, genetic diversity, and/or deleterious mutations (Frankham 2005), which may profoundly influence the expression and variability of behavior (Sutherland 1998; de Boer et al. 2018; Müller and Juškauskas 2018; Berger-Tal and Saltz 2019).

Interestingly, population subdivision and sexual selection could interactively shape the evolution of behaviors, yet this possibility has received little theoretical or empirical attention. For instance, paternity biases arising from sexually selected processes may reduce effective population sizes (Plesnar-Bielak et al. 2012; Rodriguez-Exposito and Garcia-Gonzalez 2021), thus accelerating inbreeding and loss of alleles through genetic drift in structured populations. However, sexually selected processes may also prevent the depletion of genetic variation and the spread of deleterious selfish genetic elements in subdivided populations (Price et al. 2010; Plesnar-Bielak et al. 2012). Regardless of the causal mechanism, both scenarios above are expected to have cascading effects in behavior at the individual and population level (e.g., in behavioral plasticity, learning, or foraging strategies). In addition, sexually selected processes may modulate the expression of some behaviors in subdivided populations if these behaviors are promoted under conditions of population spatial structure (Eldakar et al. 2009; Yasui and Garcia-Gonzalez 2016). However, to our knowledge, no study has explored whether a key ecological and demographic factor such as population spatial structure interplays with sexual selection in shaping behavioral evolution.

Here, by adopting a powerful approach to studying adaptation—experimental evolution—we investigate, for the first time, the independent and interactive effects of sexual selection and selection associated with metapopulation structure on the evolution of movement activity. As a model system, we used the seed beetle *Callosobruchus maculatus*, a polygamous species characterized by intense sexual selection and sexual conflict. Movement activity is a core component of critical behaviors (e.g., dispersal, predator avoidance, or resource acquisition) in a variety of taxa (Rauw et al. 2000; Réale et al. 2007; Husak and Fox 2008). Particularly, in *Callosobruchus* species, more active individuals exhibit higher metabolic rates (*C. macullatus*; Berger et al. 2014), achieve more matings (*C. chinensis*; Nakayama and Miyatake 2010a), and display different antipredator behaviors (Nakayama and Miyatake 2010a) than less active individuals, which has profound repercussions on fitness through mate or oviposition substrate acquisition and/or survival probabilities (Nakayama and Miyatake 2010b; Berger et al. 2014). Beetles from a same original population were cultured in a 2 × 2 cross-classified selection experiment, whereby binary selection treatments associated with the intensity of sexual selection (high, under a polygamous mating system, or minimal, under an enforced monogamous mating system), and associated with metapopulation structure (yes/no), were crossed. Each of the resulting four combinations of selection treatments was replicated across four populations (Rodriguez-Exposito and Garcia-Gonzalez 2021) for

79 generations. Subsequently, after a generation of common garden breeding, we measured the movement activity of individuals subjected to the four selection regimes (each combination of selection treatments) under two different environmental contexts (with and without cues from conspecifics; henceforth competitive and noncompetitive environment, respectively). The rationale behind the assays under two contrasting environments is that it is likely that individuals adjust their activity levels plastically, according to the perceived levels of competition and mating opportunities, as has been shown with other reproductive or sexually selected traits in this and other species (e.g., copulation duration or transference of seminal fluid proteins; Wedell et al. 2002; Wigby et al. 2009; Kelly and Jennions 2011; Wilson et al. 2014). Further, given that *C. maculatus* is a capital breeder (resources for maintenance and reproduction are obtained during larval development) and movement is an energetically demanding behavior (Husak and Fox 2008; Careau and Garland 2012), different evolutionary histories (e.g., polygamy vs. monogamy) could have led to different strategies regarding investment in movement activity depending on the need (e.g., noncompetitive environment vs. perceived competitive environment).
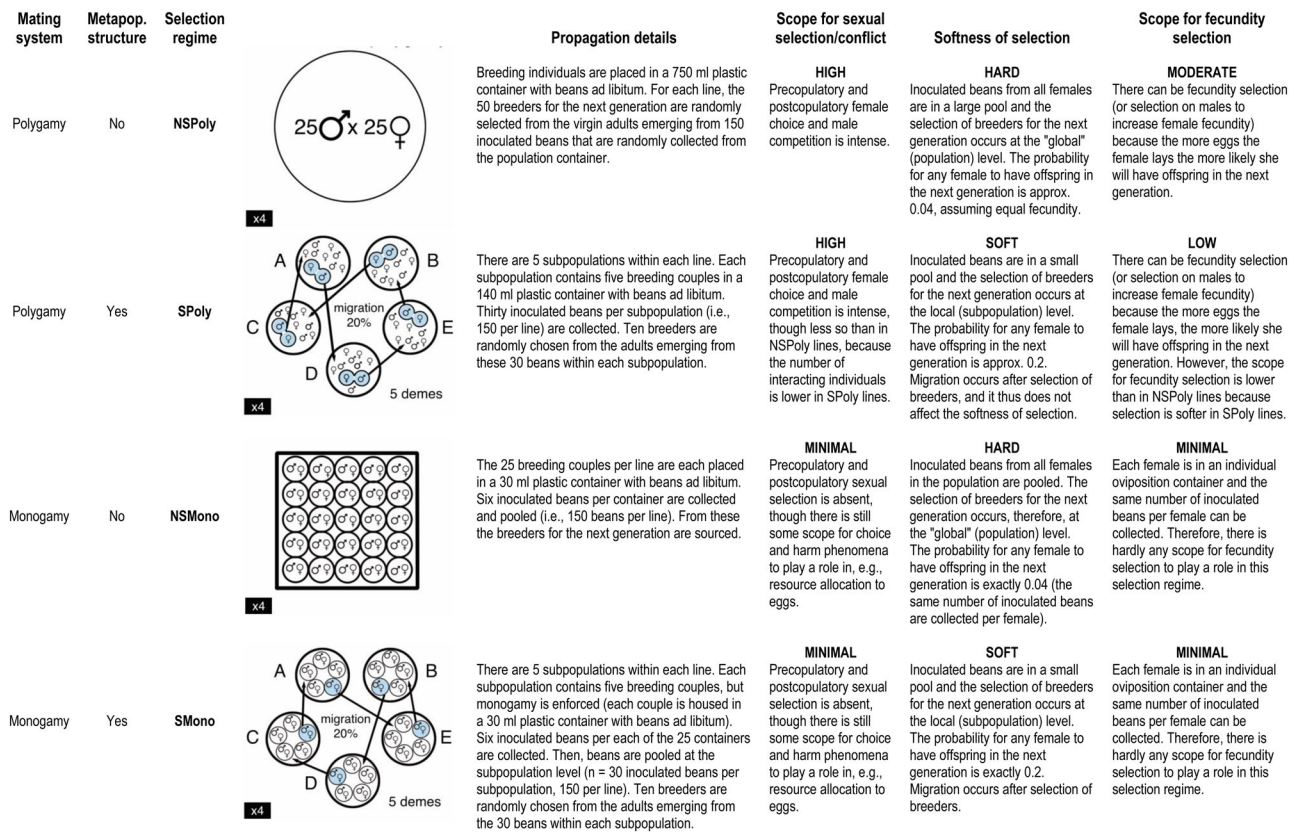
Based on previous studies on this and other species, we predict that individuals from populations with an evolutionary history of intense sexual selection and sexual conflict should be more active, at least when tested in competitive contexts (e.g., Husak and Fox 2008; Nakayama and Miyatake 2010a,b; Careau and Garland 2012; Nandy et al. 2013). Predictions on activity levels regarding population spatial structuring are unclear as there is a paucity of studies on the behavioral consequences of metapopulation structuring. However, to the extent that population subdivision leads to demes of small population sizes, metapopulation structure might favor the evolution of more active individuals with a higher propensity to disperse in search of additional mating partners (Eldakar et al. 2010), or resources. Further, we predict an interaction between mating system and metapopulation structure selection histories affecting activity. Movement activity is expected to be associated with sexual conflict (male competitiveness and ability to monopolize females, and female ability to resist male harassment and mating attempts), and recent work demonstrates that metapopulation structure reverses the patterns of sexual antagonism typically found in undivided populations (Rodriguez-Exposito and Garcia-Gonzalez 2021). In addition, the complete isolation of demes within a population may influence behaviors such as aggression in sexual conflict systems (Eldakar et al. 2009). Finally, if individuals adjust their activity plastically according to perceived levels of competition and mating opportunities, we expect an increase in movement activity and, particularly, an increase in the activity of polygamous compared to monogamous males, in environments containing cues from conspecifics.

Materials and methods

We used the seed beetle *Callosobruchus maculatus* (Chrysomelidae, Bruchinae), a popular model organism in sexual conflict studies (see Supporting Information for extended details on the study system). The study population was established in 2013 in the Estación Biológica de Doñana (Seville, Spain) with over 450 founding individuals tracing back to the original natural population in South India (Fox et al. 2003). The culture is maintained on mung beans (*Vigna radiata*) at 29°C, 40% relative humidity, and a 12L:12D cycle, using three to four population replicates (with large population sizes >300 individuals) that are mixed every few generations (Zajitschek et al. 2018; Rodriguez-Exposito and Garcia-Gonzalez 2021).

SELECTION EXPERIMENT

Full details of the selection experiment are provided elsewhere (Rodriguez-Exposito and Garcia-Gonzalez 2021). Briefly, using the source population described above, we drew 16 samples that would each generate a different selection line (see below and Fig. 1), and applied an experimental evolution protocol consisting of the combination of two selection treatments related to (i) the type of mating system, wherein individuals are allowed to mate polygamously or rather forced to mate monogamously, that is, with intense (polygamy) or relaxed (monogamy) sexual selection and sexual conflict, and (ii) the presence/absence of population spatial structure (see Fig. 1). Each of the four selection regimes was replicated across four lines, thus the selection experiment consisted of a 2 × 2 design (spatial structure × mating system treatment) with 16 selection lines in total: (i) four lines under a selection history of polygamy and absence of population structure (henceforth NSPoly lines, i.e., nonstructured polygamous lines); (ii) four lines under polygamous selection history and spatial population structure (SPoly lines, i.e., for structured polygamous lines); (iii) four lines under a selection history of monogamy in combination with the absence of population structure (nonstructured monogamous lines, henceforth NSMono lines); and (iv) four lines under monogamous and spatial population structure selection histories (structured monogamous lines, henceforth SMono lines). Selection associated with population spatial structure was created because each of the four SPoly and four SMono lines were subdivided into five subpopulations. The remaining eight populations (NSPoly and NSMono lines) were kept undivided. To prevent population subdivision in nonstructured lines (NSMono and NSPoly), the offspring of all the couples constituting each line (i.e., all the inoculated beans) was pooled before randomly selecting the virgin adults for the next generation (further details in Fig. 1, Supporting Information, and Rodriguez-Exposito and Garcia-Gonzalez 2021).

Mating system	Metapop. structure	Selection regime		Propagation details	Scope for sexual selection/conflict	Softness of selection	Scope for fecundity selection
Polygamy	No	NSPoly	25♂ x 25♀ (x4)	Breeding individuals are placed in a 750 ml plastic container with beans ad libitum. For each line, the 50 breeders for the next generation are randomly selected from the virgin adults emerging from 150 inoculated beans that are randomly collected from the population container.	**HIGH** Precopulatory and postcopulatory female choice and male competition is intense.	**HARD** Inoculated beans from all females are in a large pool and the selection of breeders for the next generation occurs at the "global" (population) level. The probability for any female to have offspring in the next generation is approx. 0.04, assuming equal fecundity.	**MODERATE** There can be fecundity selection (or selection on males to increase female fecundity) because the more eggs the female lays the more likely she will have offspring in the next generation.
Polygamy	Yes	SPoly	A B C E D migration 20% 5 demes (x4)	There are 5 subpopulations within each line. Each subpopulation contains five breeding couples in a 140 ml container with beans ad libitum. Thirty inoculated beans per subpopulation (i.e., 150 per line) are collected. Ten breeders are randomly chosen from the adults emerging from these 30 beans within each subpopulation.	**HIGH** Precopulatory and postcopulatory female choice and male competition is intense, though less than in NSPoly lines, because the number of interacting individuals is lower in SPoly lines.	**SOFT** Inoculated beans are in a small pool and the selection of breeders occurs at the local (subpopulation) level. The probability for any female to have offspring in the next generation is approx. 0.2. Migration occurs after selection of breeders, and it thus does not affect the softness of selection.	**LOW** There can be fecundity selection (or selection on males to increase female fecundity) because the more eggs the female lays, the more likely she will have offspring in the next generation. However, the scope for fecundity selection is lower than in NSPoly lines because selection is softer in SPoly lines.
Monogamy	No	NSMono	(grid of couples) (x4)	The 25 breeding couples per line are each placed in a 30 ml plastic container with beans ad libitum. Six inoculated beans per container are collected and pooled (i.e., 150 beans per line). From these the breeders for the next generation are sourced.	**MINIMAL** Precopulatory and postcopulatory sexual selection is absent, though there is still some scope for choice and harm phenomena to play a role in, e.g., resource allocation to eggs.	**HARD** Inoculated beans from all females in the population are pooled. The selection of breeders for the next generation occurs, therefore, at the "global" (population) level. The probability for any female to have offspring in the next generation is exactly 0.04 (the same number of inoculated beans are collected per female).	**MINIMAL** Each female is in an individual oviposition container and the same number of inoculated beans per female can be collected. Therefore, there is hardly any scope for fecundity selection to play a role in this selection regime.
Monogamy	Yes	SMono	A B C E D migration 20% 5 demes (x4)	There are 5 subpopulations within each line. Each subpopulation contains five breeding couples, but monogamy is enforced (each couple is housed in a 30 ml plastic container with beans ad libitum). Six inoculated beans per each of the 25 containers are collected. Then, beans are pooled at the subpopulation level (n = 30 inoculated beans per subpopulation, 150 per line). Ten breeders are randomly chosen from the adults emerging from the 30 beans within each subpopulation.	**MINIMAL** Precopulatory and postcopulatory sexual selection is absent, though there is still some scope for choice and harm phenomena to play a role in, e.g., resource allocation to eggs.	**SOFT** Inoculated beans are in a small pool and the selection of breeders for the next generation occurs at the local (subpopulation) level. The probability for any female to have offspring in the next generation is exactly 0.2. Migration occurs after selection of breeders.	**MINIMAL** Each female is in an individual oviposition container and the same number of inoculated beans per female can be collected. Therefore, there is hardly any scope for fecundity selection to play a role in this selection regime.

Figure 1. Scheme of the experimental evolution protocol with a description of the propagation of lines and an indication of the aspects of selection (intensity of sexual selection and sexual conflict, softness of selection, and scope for fecundity selection) associated to each selection regime. Variation in mating system (monogamy vs. polygamy) and variation in metapopulation structure (no vs. yes) were combined to generate four different selection regimes, each one replicated four times (16 selection lines in total), for 79 generations. All selection lines in all selection regimes were propagated with 25 breeding males and 25 breeding females, and in all selection lines sexual interactions and egg laying took place over 48 hours. The volume of the individuals' enclosures in the different treatments was adjusted to maintain an approximately constant density of individuals across treatments (see Supporting Information). In regimes under metapopulation structure, the selection line was subdivided into five different demes (subpopulations; indicated by letters A–E). To allow gene flow in the metapopulation lines, in each generation one randomly chosen individual from each sex and subpopulation (highlighted in blue) was transferred to a different randomly chosen subpopulation, so that each subpopulation received only one male-female migrant pair from another deme. The direction of the arrows connecting the subpopulations in the figure is a random representation of the migration scenario. See main text for further details.

Initial individuals for the selection experiment were randomized among groups and populations. Subsequent generations were originated and maintained with 50 breeders per line in an equal sex ratio. In all lines, sexual interactions between virgin adult individuals (1—4 days after emergence) and egg laying were allowed for 2 days (a relevant period considering the life span of the species) in an environment with beans ad libitum (a standard volume of beans was always applied per capita) to make larval competition negligible (Rodriguez-Exposito and Garcia-Gonzalez 2021). The selection protocol was maintained for 79 generations.

As some degree of gene flow among populations is a critical aspect of metapopulations (Hanski and Gaggiotti 2004), we applied a moderate migration rate for the lines subjected to spatial structure. In each generation and within each metapopulation line, 20% randomly chosen individuals from each subpopulation (i.e., one male and one female per subpopulation) were relocated to a different subpopulation. Relocations were carried out upon adult emergence using virgin individuals to introduce gene flow among subpopulations, but without altering other aspects of the selection protocol. It must be noted that metapopulations are typically defined by population subdivision and connectivity, but also by related phenomena such as deme extinctions and recolonizations, or dynamic variation in deme size. Our design is clearly a tractable simplification of real metapopulations, but still retains the most salient metapopulation features: population spatial structure and population connectivity. As mentioned above, variation in mating system was superimposed

Table 1. The results of Linear Mixed Models testing the effects of the selection treatments (polygamy vs. monogamy, and presence vs. absence of population structure) and its interaction, on the movement activity of females (a) and males (b).

(a) Females					
Random effects	σ^2				
Individual identity	8.82				
Selection line	3.312				
Batch	1.989				
Residual	22.798				
Fixed effects:	β	**SE**	**df**	**Wald χ^2**	***P*-value**
Intercept	22.558	14.883	126.962	–	–
Mating system [polygamy]	–3.933	1.870	15.557	9.227	**0.002**
Structure [yes]	0.550	1.849	15.114	0.717	0.397
Environment [competitive]	–0.327	1.039	129.989	1.258	0.262
Size	–0.122	4.504	123.527	0.002	0.964
Age	–0.910	0.837	121.957	0.855	0.355
Relative recording time within batch	–0.058	0.706	3.104	7.080	**0.008**
Mean recording time for each batch	0.147	0.877	119.348	0.105	0.746
Msystem [polygamy] × Structure [yes]	–0.048	2.436	11.454	0.003	0.955
Environment [competitive] × Msystem [polygamy]	1.348	1.170	129.975	1.306	0.253
Environment [competitive] × Structure [yes]	0.503	1.169	129.967	0.177	0.674
(b) Males					
Random effects	σ^2				
Individual identity	30.3				
Selection line	10.66				
Batch	0				
Residual	16.19				
Fixed effects:	β	**SE**	**df**	**Wald χ^2**	***P*-value**
Intercept	51.158	21.087	124.404	–	–
Mating system [polygamy]	–0.707	2.949	14.763	2.052	0.152
Structure [yes]	2.864	2.866	13.549	1.365	0.243
Environment [competitive]	–1.574	0.835	134.092	0.280	0.597
Size	–16.401	8.635	108.185	3.775	**0.052**
Age	–0.181	1.156	124.487	0.046	0.831
Relative recording time within batch	–1.593	0.263	146.924	37.584	**0.000**
Mean recording time for each batch	0.439	1.204	123.710	0.112	0.738
Msystem [polygamy] × Structure [yes]	–3.777	3.993	12.815	1.172	0.279
Environment [competitive] × Msystem [polygamy]	0.574	0.969	134.096	0.362	0.548
Environment [competitive] × Structure [yes]	2.102	0.970	134.107	4.838	**0.028**

Individual activity was measured in two different environments: competitive and noncompetitive. Bold values denote statistical significance at the $P < 0.05$ level. See *Methods* for further details.

onto this design. In the eight polygamous lines, interactions and matings were allowed without restriction among individuals in the population (for lines without population structure; NSPoly lines), or in the subpopulation (SPoly lines). In contrast, in the eight monogamous lines (NSMono and SMono lines), interactions and matings occurred on a one male-one female basis, by establishing pairs with randomly collected individuals from within the population (NSMono) or subpopulation (SMono). Although there is some scope for females to allocate resources to eggs depending on male traits (including those related to mate harming)

in the monogamous lines, the enforced monogamous treatment prevents pre- and postcopulatory female choice of mates, and male-male competition, and thus minimizes sexual selection and the opportunity for sexual conflict (Fig. 1).

Effective population size did not differ greatly across selection regimes (Rodriguez-Exposito and Garcia-Gonzalez 2021). Furthermore, evidence gathered thus far from our selection lines indicates little scope for differences in inbreeding or drift in our selection experiment as there are no differences in fertility rates, baseline longevity, or reproductive rates among selection regimes

(Rodriguez-Exposito 2018; Rodriguez-Exposito and Garcia-Gonzalez 2021). Therefore, any observed change in activity levels can be attributed to evolutionary responses to the selection treatments independent of effective population size, inbreeding, or drift. These responses are likely related to the extent and number of sociosexual interactions (each individual can interact with another 49 individuals in NSPoly lines, nine individuals in SPoly lines, or only one individual in NSMono and SMono lines), or to the softness of selection (hard selection occurs in NSPoly and NSMono lines, whereas soft selection operates in the structured lines) (see Fig. 1, Supporting Information; Wallace 1975; Saccheri and Hanski 2006; Débarre and Gandon 2011; Reznick 2016; Bell et al. 2021; Li Richter and Hollis 2021).

The behavioral assays (detailed below) were performed after one generation of common garden, wherein individuals from all lines were bred under polygamous conditions and absence of spatial structure, that is, we established 16 replicated common garden lines, all under NSPoly conditions. As a result, any divergence in movement activity could be attributed to genetic effects (i.e., evolutionary effects genetically assimilated) rather than to environmental effects (e.g., maternal or paternal effects) associated to the type of breeding or the particular conditions (Kawecki et al. 2012).

BEHAVIORAL ASSAYS

At day 2–3 after emergence, we measured movement activity in virgin individuals (sample sizes for each selection regime are shown in Table S1 in Supporting Information). *Callosobruchus maculatus* beetles typically move up then fall down along the wall of their home containers. This behavior exhibits a considerable repeatability in the short term (across different 10-minute batches: $R = 0.62$) and long term (across 3 days of difference: $R = 0.43$; Canal et al. 2021). Previous work also shows that movement activity measured in similar confined spaces is a good proxy of other important functional traits in *Callosobruchus* species (metabolic rate, mating success, and antipredator behavior; Nakayama and Miyatake 2010a; Berger et al. 2014).

To assess movement activity, we placed each individual in a separate glass tube (3.8 cm high, 1 cm diameter), which were then aligned vertically in front of a high-resolution camera, and recorded individuals for 45 minutes. The camera was connected to the software Ethovision 12XT (Multiple Arenas Module; Noldus Information Technology, Wageningen, the Netherlands), which calculates the absolute movement (in cm) of each recorded individual. We discarded the first 5 minutes of recordings to negate residual effects of handling and the presence of the experimenter in the vicinity. We simultaneously recorded 12 individuals, arranged along a 2 × 6 (rows by columns) setup. Within the 2 × 6 setup, individuals were randomly positioned with respect to the selection regime, beetles were always introduced and removed from the tubes in the same order (from position 1 to 12), and the position of the tubes was considered in the statistical analyses. As a control, we always added one tube containing a dead animal to calibrate, if needed, the minimum of movement distance between two consecutive video frames, thus ensuring that immobile individuals were truly recorded with zero distance. To avoid overestimation of distance due to falls along the walls of the tubes (not considered as true moved distances), we also set the maximum distance moved between two video frames. To this end, we recorded the maximum distance moved between two consecutive frames as well as the distance moved during the falls in a subset of individuals. As the latter was higher than the former, we could set a threshold to reliably exclude the falls from the raw measurements (see Canal et al. [2021] for further details).

We assessed the activity of each virgin individual twice, in assays that simulated two different environments with regard to the presence of cues for competition: (i) in a new glass tube, not used before by any other individual ("noncompetitive environment") and (ii) in a glass tube within which eight (four males and four females) tester individuals were allowed to move and interact freely during the 30 minutes immediately preceding the recordings ("competitive environment"). Tester individuals were randomly sourced from a large container containing several hundred individuals belonging to a pool of individuals from all the lines. The tester individuals used were different for each focal individual. Before starting the assays, the tester individuals were removed from the tubes, thus focal individuals were recorded alone, but in an environment with abundant chemical information on the presence of conspecifics. Focal individuals were recorded once per environment and both recordings were conducted using the same kind of container (glass tubes) and the same recording settings (the same 2 × 6 platform and light conditions). To control for an acclimation and/or learning effect (Dukas 2006), we randomized the order of the recordings among individuals (which was carried out blindly with respect to selection line and regime). Therefore, some individuals were first recorded in the noncompetitive environment, whereas others were first recorded in the competitive environment (i.e., noncompetitive → competitive or vice versa). Although the two recordings per individual were conducted in the same day, all focal individuals scheduled for the day were recorded first in one environment (either in the competitive or noncompetitive environment) before starting the second round of assays (in the remaining environment), that is, we had two batches of records (early and late; see statistical analyses below). All the behavioral assays were conducted between 9:00 a.m. and 3:00 p.m. and time was included in the models to account for potential fluctuation in daily biorhythms. Assays were conducted at similar conditions of temperature and humidity to those used to maintain the stock population.

Our data are not feasible to separate within- and among-individual correlations on movement activity because we do not have repeated measures of the behavior at different life stages. The interpretation of our results must thus rely on the assumption that the assessed behavioral trait reflects individual-specific strategies that are preserved throughout life ("individual gambit"; Brommer 2013). However, three important points need to be taken into account. First, the age (2–3 days old) at which behavioral assays were conducted coincides with the period in which individuals were housed with the other sex throughout the selection experiment and oviposition took place. Thus, behavioral measurements were made in the most relevant period for fitness maximization (reproductive competition, oviposition decisions, etc.) in the context of our study. Second, previous work on this system indicates that within-individual changes in movement activity at different ages (on the 2nd and 4th day of adult life) are similar among individuals, which means that there is no change in the ranking of individuals according to their activity (Canal et al. 2021). Third, if important differences (unmeasured in our study) were to emerge later in life, we might not have detected behavioral changes early in life (see *Results*), because within-individual variance is more likely to mask, rather than exacerbate, differences between groups in this highly controlled experimental situation.

Body size was estimated, blind with respect to line ID, from elytron length following the methods of Rodriguez-Exposito and Garcia-Gonzalez 2021) and included in the statistical models to account for potential differences in movement distance due to this trait (Beukeboom 2018).

STATISTICAL ANALYSES

We used linear mixed models (LMMs) to investigate the effects of the selection regimes on individual activity. We ran the analyses for each sex separately for two reasons. First, males and females have contrasting life histories (e.g., reproductive strategies and trade-offs; Fox et al. 2003; Rönn et al. 2007; Hotzy and Arnqvist 2009; Berger et al. 2014). Second, exploratory analyses suggested different patterns of activity between sexes because (i) males were more active than females regardless of the environment and the selection treatment (LMM: $\chi^2 = 4.75$, $P = 0.029$), (ii) the three-way interactions between sex, mating system, and population spatial structure and those involving sex, environment, and selection treatment (either mating system or structure) were nonsignificant (all $P > 0.3$), and (iii) the within-individual correlation of movement activity across the two assays was twofold higher in males than females (Pearson correlation: males: $r = 0.67$, $P < 0.001$, females: $r = 0.34$, $P < 0.001$).

In the activity models, one for each sex, the distance moved (square-root transformed, see below) was included as the response variable, whereas mating system (monogamy/polygamy), population spatial structure (yes/not), environmental context (noncompetitive/competitive), as well as the two-way interactions between environmental context × mating system, environmental context × structure, and mating system × structure were included as predictors. Further, body size, age at the time of behavioral assay (2–3 days old), relative time within the batch (time centered within each batch; i.e., within-batch effect), and the mean time of the records for each batch (i.e., between-batch effect) were included as control variables. Selection line identity (a unique code for each of the 16 selection lines), individual identity (each individual is tested in two behavioral assays), recording batch (early, late), and the position of the tube within the recording setup wherein individuals were recorded were initially included as random factors in the model. However, the variance explained by the position of the recording tube was zero; thus, this variable was excluded from further models.

Before interpreting any model outcome, we systematically performed data exploration and several model diagnostic statistics (e.g., model residuals, multicollinearity, and the effect of influential data points; Zuur et al. 2010; Loy and Hofmann 2014) to avoid misleading results based on statistical artifacts. Based on these diagnostics, movement distance was square-root transformed. After this transformation, the diagnostic analyses did not show obvious deviations from the assumptions of linear models. Statistical analyses were performed using R version 3.6.2 (R Core Team 2019). For the mixed modeling, we used the package *lme4* (Bates et al. 2014). Significance of the fixed effects in the models was calculated with Type II (Type III in the presence of significant interactions) Wald Chi-Square tests, using the function Anova (*car* package; Fox and Weisberg 2011) on maximum likelihood models, whereas parameter estimates were extracted from Restricted Maximum Likelihood models, as suggested (Zuur et al. 2009). The package *HLMdiag* (Loy and Hofmann 2014) and the VIF function (*car* package) were used for model diagnostics.

Results

In total, we measured the movement activity of 288 individuals (143 females and 145 males from four selection regimes; 18 individuals per line, involving between seven and 11 individuals of each sex; see Table S1) in two environmental contexts.

Females evolving under a monogamous mating system were more active than those under the polygamous treatment and this pattern remained in both environmental contexts after taking into account the effect of recording time on activity (Table 1; Fig. 2). Metapopulation structure did not affect female activity, regardless of the environmental context (Table 1; Fig. 2). The interaction between mating system and population spatial structure did not influence movement activity in females (Table 1; Fig. 2).

Unlike females, male activity was similar between mating systems (Table 1; Fig. 2), but their movement activity was
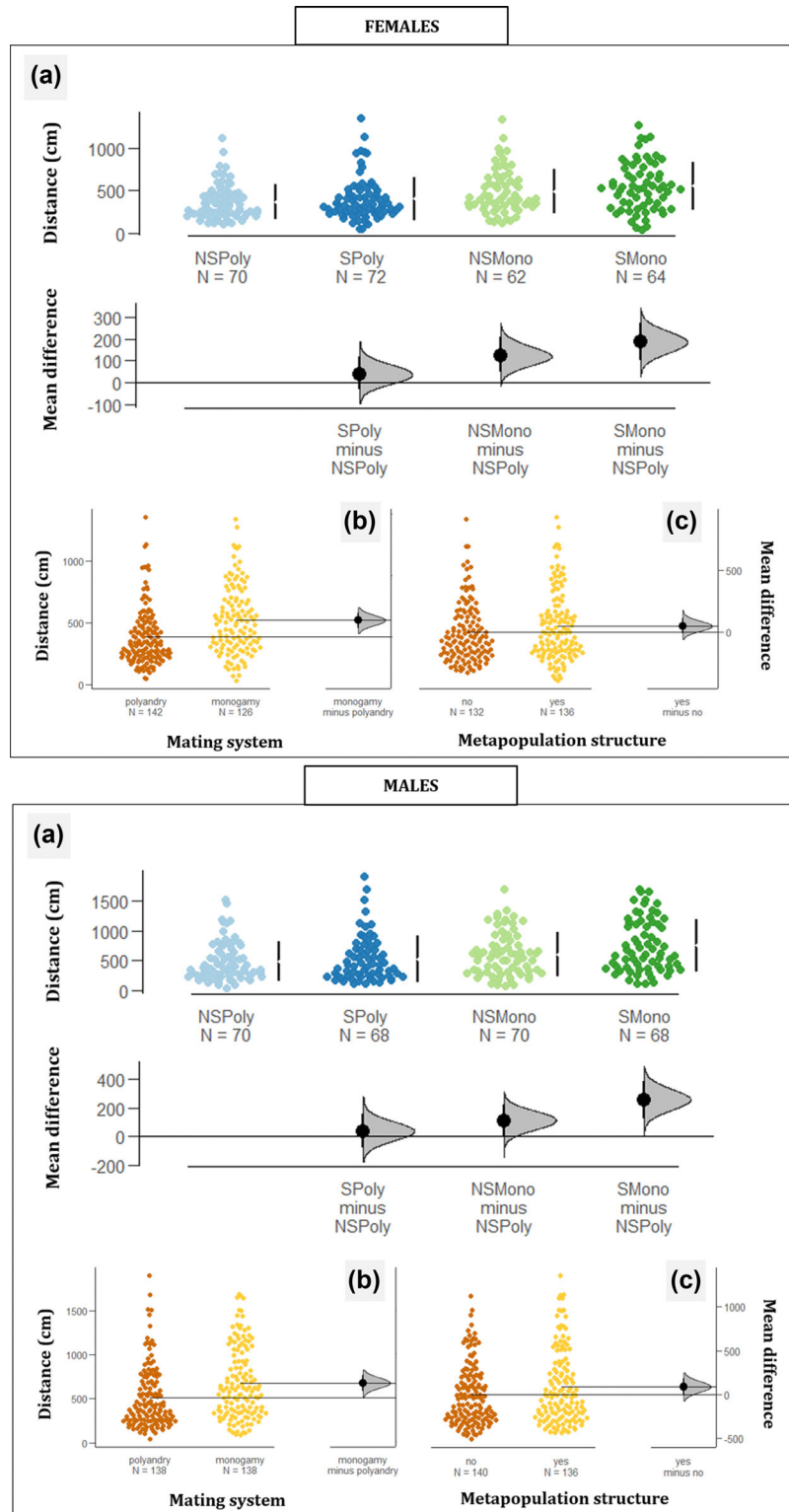
Figure 2. Variation in movement activity (walked distance, cm) for females (top graph) and males (bottom graph). (a) Movement activity in relation to the four selection regimes NSPoly (polygamy in absence of metapopulation structure), SPoly (polygamy and metapopulation structure), NSMono (monogamy in absence of metapopulation structure), and SMono (monogamy and metapopulation structure). (b) Movement activity in relation to the mating system (monogamy/polygamy) and (c) to the presence (yes/no) of metapopulation structure (see *Methods*). In each figure, the raw data are represented by colored dots while the mean difference in movement activity versus the control regime (a: NSPoly -polygamy and no structure-, b: polygamy, c: no spatial structure) is shown in a Cumming estimation plot (bottom part in figure a, right part in figures b and c). Mean differences are plotted as bootstrap sampling distributions. Each mean difference is depicted as a dot, whereas 95% confidence intervals are indicated by the vertical error bars.
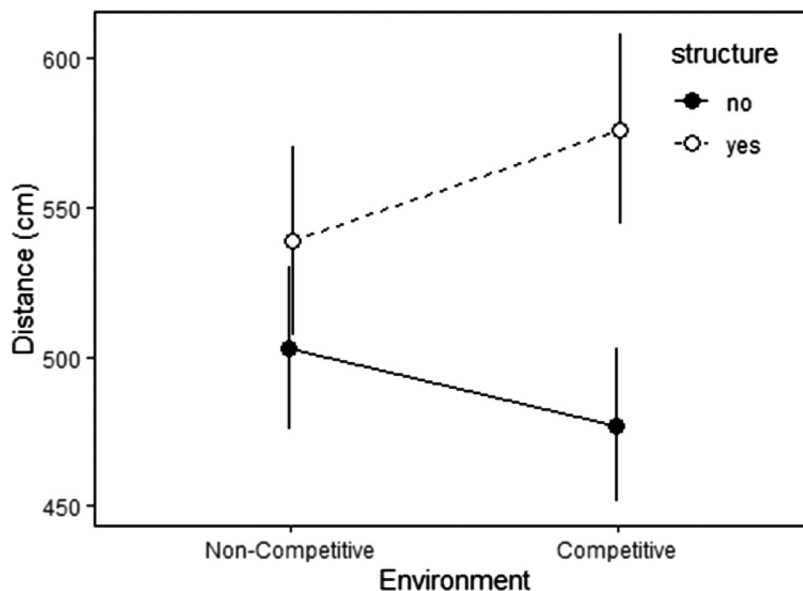
Figure 3. Interactive effect of metapopulation structure experimental evolution treatment (structure vs. no structure) and competition environment (competitive vs. noncompetitive environment) on males' movement activity. Error bars reflect standard errors around the group specific means.

affected by the interactive effects of population structure and the environmental context (after controlling for the effects of body size and recording time on activity). In the competitive environment, males from populations with a selection history associated with population subdivision increased their activity relative to that recorded in the noncompetitive environment, whereas males evolving in the absence of metapopulation structure decreased it (Table 1; Fig. 3). The interaction between mating system and population spatial structure did not affect male activity (Table 1; Fig. 2).

Discussion

We used experimental evolution to investigate the independent and interactive effects of sexual selection intensity (polygamy/monogamy) and metapopulation structure (presence/absence) on the evolution of movement activity levels in both males and females. Interestingly, contrasting selection histories led to differential evolutionary trajectories between sexes. In particular, evolution following the relaxation of sexual selection favored an increase in female movement activity, which was consistent between environmental (competitive vs. noncompetitive) contexts, whereas no such response was detected in males. In contrast, selection associated with the presence/absence of metapopulation structure had no effect on female behavior, whereas it led to context-dependent responses in male activity. All the assays were conducted after one generation of common garden. Our results provide direct experimental evidence that sex-

ual selection and population spatial structure are independent, and not interactive, important factors in the evolution of individual locomotor activity, and that they impact this behavior differently in each sex.

Experimental evolution has rarely been used to investigate the evolution of activity levels (Nandy et al. 2013; Hollis and Kawecki 2014). Using this experimental approach, we have shown that females evolving under monogamy were more active than females from polygamous lines, a pattern that was consistent between assays. *Callosobruchus maculatus* exhibits intense sexual selection and conflict, which has led to striking adaptations in males and females and to sexually antagonistic coevolution (e.g., involving female-harming male traits such as spiny genitalia, and female resistance traits such as thick reproductive tract walls; Rönn et al. 2007; Hotzy et al. 2012; Berger et al. 2016; Dougherty et al. 2017). Empirical work, including studies on *C. maculatus*, has demonstrated that in species whose mating systems are dominated by sexual conflict the removal of sexual selection favors a de-escalation of sexual antagonism (Holland and Rice 1999; Wigby and Chapman 2004; Crudgington et al. 2010; Cayetano et al. 2011; Gay et al. 2011; Hollis et al. 2019; McNamara et al. 2020; Rodriguez-Exposito and Garcia-Gonzalez 2021). In our selection experiment, females from monogamous populations evolved to be more active than females from polygamous populations. Our results, therefore, do not support the notion that female activity is positively associated with female resistance to male harm (e.g., if more active females were more successful in avoiding male harassment), but rather

suggest that, conversely, more active females may be a greater risk of encountering males. Other possible explanation for our findings is that increased investment in sexually selected or resistance traits may have constrained movement activity of females evolving under polygamy. In contrast, as females evolving under monogamy are expected to have reduced mating costs, allocation in structural or physiological resistance traits would be rendered redundant, allowing them to increase their investment in other fitness-related functions (e.g., Roff 2001; Wolf et al. 2007), such as traits related to optimizing the finding of oviposition resources.

We found that activity in males was similar between mating systems. This is contrary to the prediction that sexual selection should shape movement activity in males. Reproductive competition is expected to promote the evolution of adaptations that confer a mating advantage, such as the evolution of vigorous and/or explorative males (Nakayama and Miyatake 2010a; Husak and Fox 2008; Careau and Garland 2012), which involves high activity levels (Réale et al. 2007; Nakayama and Miyatake 2010a,b). Nevertheless, similar to that found here, Berger et al. (2014) found that activity in *C. maculatus* males did not respond to artificial bidirectional selection on life span, unlike what occurred in females (as was also the case in our experiment; see above). A potential explanation is that strong sexual selection in *C. maculatus* has depleted genetic variance in male movement activity, which could explain the lack of response to different selection regimes of this trait as well as the low, if any, covariation between male and female activity in the species (Berger et al. 2014). A second possibility that could explain the similar levels of activity between males coming from different mating systems is that polygamy primarily favors traits closely related to fertilization success such as large body sizes, harmful genitalia, or larger ejaculates (e.g., Rönn et al. 2007; Simmons and Garcia-Gonzalez 2008; Cayetano et al. 2011; Hotzy et al. 2012), which could constrain activity evolution.

Given that *C. maculatus* is a capital breeder and movement is an energetically demanding, and likely context-dependent, behavior (Husak and Fox 2008; Careau and Garland 2012), an interesting possibility is that polygamous males are more strategic than monogamous males regarding investment in movement activity. Under this explanation, we would expect no differences in activity levels between polygamous and monogamous males when there are no prospects of mating (i.e., the noncompetitive environment), but increased activity levels for polygamous males (compared to their monogamous counterparts) when mating opportunities arise and male-male competition is likely (i.e., as perceived in the competitive environment). Contrary to this expectation, male activity was similar between environmental assays. We cannot rule out that our competitive environment failed to be perceived as such. Not only chemical cues but also physical interactions, absent in our trials, may be needed to effectively

stimulate higher activity levels in males. However, we think it is unlikely that chemical cues released by eight individuals immediately before assaying focal individuals failed to induce a perception of competition, especially when cuticular hydrocarbons are known to function as cues to recognize (and respond to) competitors in *C. maculatus* (Lymbery and Simmons 2019). Further, we also note that we found an interactive effect between environment and population spatial structure (see below), which indicates that our manipulation of environmental conditions was effective. Evidently, it is also possible that our tests were sensitive enough and that sexual selection fails to induce a response in activity levels for other reasons to those contemplated here.

Theoretical and empirical works have demonstrated that metapopulation structure or population subdivision modulates the intensity of sexual selection and conflict, which subsequently impacts the behavior of individuals (Eldakar et al. 2009; Yasui and Garcia-Gonzalez 2016; Lymbery et al. 2020; Rodrigues et al. 2021; Rodriguez-Exposito and Garcia-Gonzalez 2021). Surprisingly, despite its potential implications, the interactive effect of these forces in behavioral evolution has been overlooked. We recently unveiled far-reaching consequences of this interaction on sexual conflict dynamics in this study system, such that selection arising from metapopulation structure reversed the patterns of sexually antagonistic coevolution traditionally found in undivided large populations (Rodriguez-Exposito and Garcia-Gonzalez 2021). There is, therefore, scope for the interaction between mating system and population spatial structure to modulate behavioral variation, but we failed to find such effects in the present study. This result may be explained by the reasons discussed above, for example, activity may not be a sexually selected trait in the study system, although we see this possibility as unlikely, at least for males (see above). Future work expanded to other fitness-related and sexually selected behaviors is required to disentangle the evolutionary role that the interactive effects of sexual selection and key demographic factors, such as population subdivision, may play in individual behavior.

Although there were no interactive effects with sexual selection, we detected an independent influence of metapopulation structuring on male activity that was context dependent. Males evolving under spatial structure showed increased levels of activity in competitive environments compared to those assayed in noncompetitive environments, whereas the reverse was true for males from undivided populations (Fig. 3). In our selection experiment, the degree of among-individual interactions in polygamous lines with spatial structure (SPoly: five males and five females per subpopulation) was more limited compared to that allowed in polygamous panmictic lines (NSPoly: 25 interacting individuals from each sex). However, because the context-dependent effect detected indicated differences between structured and nonstructured populations (i.e., independently of

mating system treatment), the levels of individual's interactions per se cannot explain the environment-by-structure interaction. A possibility is that the behavioral divergence that we have exposed responds to the differential softness of selection between the structured (where soft selection applies) and nonstructured (where hard selection operates) populations (see Fig. 1, *Methods*, Supporting Information; Wallace 1975; Saccheri and Hanski 2006; Débarre and Gandon 2011; Reznick 2016; Bell et al. 2021; Li Richter and Hollis 2021). If this was the case, soft selection in structured populations would have allowed the evolution of plasticity in male behavior. Another possibility explaining the interaction that we unveiled could be that selection associated to the presence/absence of spatial structure generates differences in males' (but not females') cognitive aspects, as has been shown to occur in response to sexual selection in *C. maculatus* (Baur et al. 2019). If so, metapopulation structure could affect the ability to perceive and/or respond to the olfactory cues produced by conspecifics, with a subsequent impact on activity. To our knowledge, no study has investigated the potential links between population spatial structure and the evolution of animal cognition, which would be a fruitful area for future research. Another potential explanation is that the degree of genetic relatedness among individuals modulated male behavior, as kin selection is expected to relax sexual conflict (Rankin 2011; Carazo et al. 2014; Łukasiewicz et al. 2017; Lymbery and Simmons 2017, but see Faria et al. 2020). Relaxation of sexual conflict could then affect male activity levels, as reported for other traits (Rankin 2011; Carazo et al. 2014; Le Page et al. 2017; Lymbery and Simmons 2017), with this process being more evident when individuals perceive the presence of conspecifics (i.e., in the competitive environment). We are cautious, however, about this possibility for two reasons. First, contrary to the expectation that kin selection modulates male-male competition and sexual conflict, we found that males from structured populations (if anything expected to be more related than males in panmictic populations), increased (not decreased) activity under competitive conditions. Second, we imposed moderate migration rates (20%) in the metapopulation lines, and thus our settings precluded to a large extent genetic structuring (Rodriguez-Exposito and Garcia-Gonzalez 2021). On this note, we also emphasize that we imposed equal and controlled migration rates for both sexes among subpopulations, nullifying any effects of sex-biased dispersal on our results (Eldakar et al. 2009; Faria et al. 2020; Lymbery et al. 2020).

In conclusion, we have experimentally investigated the interactive effects of sexual selection and metapopulation structure on the evolution of a behavioral trait, locomotor activity, in both males and females. Although no interactive effects of mating system and population subdivision on activity were detected, we found that evolution under contrasting selection regimes led to sex- and context-dependent divergence on this behavior. Our study contributes to the limited body of research on movement activity evolution, not only in males but also in females, in response to selection arising from reproductive competition and, in addition, arising from metapopulation structuring. Research involving other key behavioral traits (e.g., male aggressiveness, female escape behavior) and a broader focus on within-individual variation is warranted to shed further light on the effects of sexual selection and population subdivision upon behavioral evolution.

DATA ARCHIVING

The data related to this study are archived in Dryad (https://doi.org/10.5061/dryad.9w0vt4bh6).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Number of individuals tested for each replicated population and selection regime.