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Evolution of sociability by artificial selection

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There has been extensive research on the ecology and evolution of social life in animals that live in groups. Less attention, however, has been devoted to apparently solitary species, even though recent research indicates that they also possess complex social behaviors. To address this knowledge gap, we artificially selected on sociability, defined as the tendency to engage in nonaggressive activities with others, in fruit flies. Our goal was to quantify the factors that determine the level of sociability and the traits correlated with this feature. After 25 generations of selection, the high-sociability lineages showed sociability scores about 50% higher than did the low-sociability lineages. Experiments using the evolved lineages indicated that there were no differences in mating success between flies from the low and high lineages. Both males and females from the low lineages, however, were more aggressive than males and females from the high lineages. Finally, the evolved lineages maintained their sociability scores after 10 generations of relaxed selection, suggesting no costs to maintaining low and high sociability, at least under our settings. Sociability is a complex trait, which we currently assess through genomic work on the evolved lineages.

KEY WORDS: **Aggression, artificial selection, *Drosophila melanogaster*, mate choice, sociability, social behavior.**

Social behavior, broadly defined as interactions among conspecifics, has attracted substantial research effort for a long time (Allee 1938; Tinbergen 1953; Wilson 1975; Clutton-Brock 2016; Ward and Webster 2016). Some minimal social activity occurs in most animals as it is typically essential for acquiring mates. In the relatively small proportion of animals that engage in parental care, individuals may also participate in parent-offspring and sibling interactions. Relatively few animals, however, live in groups, and that fraction of species has been the focus of most studies on social behavior. Notable long-term studies on such highly social species include work on the social behavior of ants, wasps, and bees (Wilson 1971; Michener 1974; Seeley 2010; Kapheim et al. 2015), social mammals including naked mole rats (*Heterocephalus glaber*) (Jarvis 1981; Sherman et al. 1991; Barker et al. 2021), elephants (*Loxodonta africana*) (Moss et al. 2011) and primates (Goodall 1986; Cheney and Seyfarth 2008; Clutton-Brock 2016), and cooperatively breeding birds (Brown 1987; Koenig and Dickinson 2004).

Although the research on animal societies has been illuminating, there has been increased recognition that apparently solitary species engage in persistent social interactions outside the obvious realms of brief encounters in the context of courtship and mating (Caro 1994; Costa 2006). For example, recent work on a classical solitary, territorial mammal, the puma (*Puma concolor*), has indicated that every individual participated in a dense social network, with animals routinely sharing their kills with other individuals (Elbroch and Quigley 2017; Elbroch et al. 2017). The evidence for complex social behaviors in apparently solitary species suggests that we can gain insights about the evolutionary biology of social behavior by focusing on animals traditionally classified as nonsocial.

A key evolutionary model species, the fruit fly (*Drosophila melanogaster*), had been historically classified as nonsocial. Although hints of fruit flies' social behavior existed for a long time, much of the research on that topic is recent. The discovery that *cis*-vaccenyl acetate (cVA) serves as an aggregation pheromone of fruit flies (Bartelt et al. 1985) implied social attraction, which led to research on its adaptive significance (Wertheim et al. 2002). Further research has documented social synchronization of the circadian clock (Levine et al. 2002), social learning (Sarin and Dukas 2009; Battesti et al. 2012), the formation of social groups (Saltz 2011; Schneider et al. 2012; Simon et al. 2012;

Anderson et al. 2016; Scott et al. 2018; Bentzur et al. 2021), and collective response to danger (Ramdya et al. 2015; Ferreira and Moita 2020).

Although social behavior includes many features, we focus here on a key trait, sociability, defined as animals' tendency to engage in nonaggressive activities with conspecifics. Such activities may include feeding together, traveling in a group, and communal resting or sleeping. Sociability means that individuals either seek each other, tolerate other members of a group, or often both. Field and laboratory studies indicate that both larval and adult fruit flies show significant sociability, as they prefer to group together at food patches (Durisko et al. 2014; Anderson et al. 2016; Scott et al. 2018; Dukas 2020). In the adults, the broad sense heritability of sociability is about 0.22 (Scott et al. 2018). The heritable variation in sociability opens up exciting opportunities for assessing the evolutionary biology of this trait in a prominent model animal. Specifically, we were interested in quantifying costs and benefits of sociability as well as its genetic correlation with other fitness-relevant traits. To this end, we artificially selected on low and high sociability for 25 generations.

Given the heritable variation in sociability, we predicted that we would succeed in generating diverged low- and high-sociability lineages. We then focused on four key predictions tested on the evolved lineages. First, we predicted that flies from the low and high lineages would vary in their mating success. We expected lower mating success of males from the high than low lineages as we expected them to be more docile in their interactions with females. For the females, however, we had no a priori rationale for a directional prediction. Second, we predicted that flies from the low lineages would be more aggressive than flies from the high lineages. Intuitively, it is sensible to assume that the tendency to share a small food patch with others would be negatively associated with aggression. Nevertheless, the genetic correlation between sociability and aggression may be complex given that aggression is often necessary for establishing dominance in social groups.

Our third prediction implicated unknown likely costs of possessing sociability scores below and above those expressed by the baseline wild population. We thus predicted that 10 generations of relaxed selection would lead to convergence in the sociability scores of the low and high lineages. Finally, as noted above, social behavior comprises many features. Although we focused on individuals' tendencies to seek and tolerate others at a small food patch, one can measure other potentially relevant traits. One such trait is the nearest neighbor distance (NND), which indicates how tolerable individuals are to other proximate individuals (Conder 1949; Marler 1956). Given the likely positive association between sociability and NND, we predicted a larger NND in the low than high lineages.

Methods

ESTABLISHING STARTING POPULATION AND SELECTION AND CONTROL LINEAGES

We derived all artificial selection lineages from a population of ∼600 wild *Drosophila melanogaster* females caught in various locations in and around Hamilton, Ontario in late spring and early summer 2018. We transferred each female into a standard food vial (1 L standard food = 90 g sucrose, 75 g cornmeal, 10 g agar, 32 g yeast, and 2 g methyl paraben dissolved in 20 mL ethanol), and we verified the species based on male morphology in F1 progeny. We chose to use a freshly wild caught population over a lab-adapted population to maximize ecologically relevant genetic variation available for selection. A caveat with this approach, however, is that lab adaptation occurs simultaneously with artificial selection, potentially reducing the effectiveness of our selection regime.

We mixed three F1 males and three F1 females from each of these isofemale lines together in three large populations. We then amplified these populations over one or two generations, generating a large total population size of ∼6000 flies, mixed among the three populations, and then randomly assigned flies to 12 separate lineages: four lineages to be selected for low sociability, four lineages to be selected for high sociability, and four control lab adaptation and domestication lineages. The control lineages were not involved in the present experiments, and are used as controls in ongoing genomic and gene expression analyses. We housed each lineage in a population cage ($20 \times 20 \times 30$ cm^3) with standard food bottles for one generation (∼150 males and 150 females per cage), with their offspring being the first generation subjected to artificial selection. Once selection began, we maintained the lineages in vials, as described in the detailed selection methods section below.

ORIGINAL SOCIABILITY SELECTION ARENA

We developed a novel arena capable of both quantifying the sociability of groups of flies and allowing for the selection of flies based on their sociability (Fig. 1A). We used polystyrene Petri dishes (90-mm wide × 20-mm high) as the base of each arena, with 1.5-mm-thick opaque white polystyrene dividers permanently fused to the inside of the dish. The dividers separated the interior of the arena into eight equally sized radial compartments that converged on a ∼16-mm-wide central area in the middle of the dish. Openings ∼5-mm wide allowed access to each compartment from the central area.

We built the lids out of square pieces of 3-mm-thick acrylic, as the stock Petri dish lids are not sufficiently flat to prevent fly movement over the dividers. We drilled two 16-mm-wide holes in each lid (Fig. 1B), one located centrally to allow aspirating

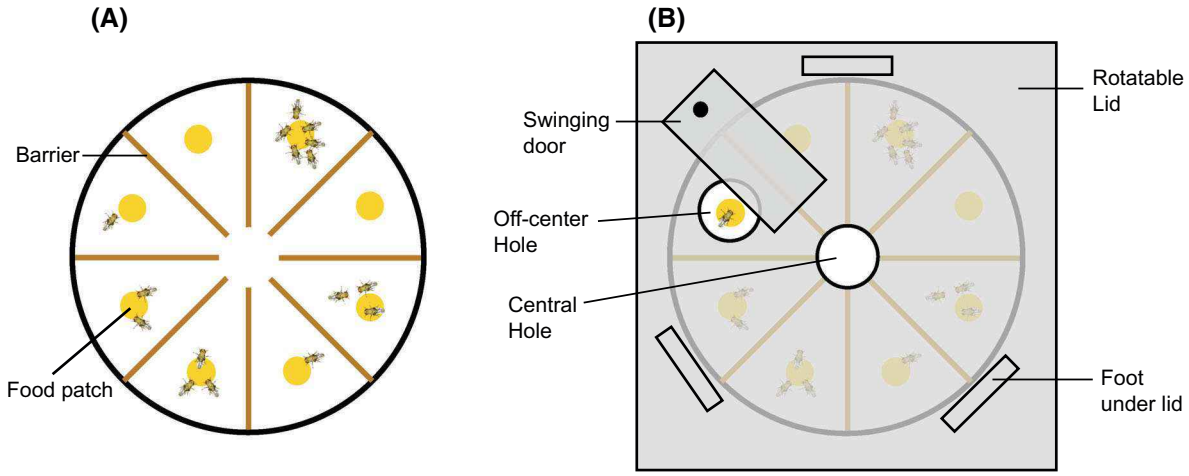

Figure 1. **The arena used for quantification of and artificial selection on sociability. (A) The arena without the lid, showing the eight compartments and an example arrangement of 16 flies. (B) The arena with the lid (note that the lid and swinging door were fully transparent, and opacity in the diagram is only for clarity). A foam plug at the central hole (not shown in the figure) allowed fly movement among the eight compartments when at the top position, and locked flies within their compartment when in the bottom position.**

flies into the central area of the arena, and one off-center directly above a compartment to allow aspirating out selected flies. We also added small strips of acrylic to the underside of the lid to act as guides that allowed the lid to remain in position while we rotate the lid so that the off-center hole could be above any compartment. We bolted a small piece of rectangular 3-mm-thick acrylic to the surface of the lid above the off-center hole, which acted as a swinging door (Fig. 1B). We used 25.4-mm-thick foam cylinders as plugs for the central hole in the lid, with 16-mm-wide plastic circles hot-glued to the bottom of the foam and coated with a slippery substance (Surfasil, ThermoFisher, Ottawa, ON, Canada) to deter flies from standing on the foam, which was evident during preliminary testing.

Before adding flies to an arena for testing, we added small discs of standard food (7-mm wide × ∼2-mm thick) coated with a layer of grapefruit-yeast solution (3 g yeast dissolved in 100 mL grapefruit juice) to the center of each of the eight compartments (Fig. 1A). We then fit the foam plug so that just the central hole in the lid was plugged, but the entrances to the compartments were fully open and thus allowing free fly movement.

REVISED SOCIABILITY SELECTION ARENA

Our observations during the first 10 generations of selection suggested that the arenas were too large, allowing individuals to be effectively socially isolated from each other within a single compartment. Hence, starting at generation 11, we switched to smaller arenas while maintaining an identical design (Fig. 1). We made each small arena from cut sections of PVC tubing 47.5 mm in diameter and 7-mm high glued to an acrylic base. We used 0.75-mm-thick polystyrene dividers, making the gaps between entrances to each chamber ∼4 mm and the central area ∼12 mm in diameter. The dimensions of the food disc remained the same. We implemented the new arena starting in generation 11 for males and generation 12 for females.

OVERVIEW OF ARTIFICIAL SELECTION METHODS

Overall, each generation, we tested 12 groups of 16 males and 12 groups of 16 females from each of the eight selection lineages (four low sociability, four high sociability). We selected four flies from each group of 16 flies to produce the next generation. In tests involving the low-sociability lineages, we chose the least sociable flies. In tests involving the high-sociability lineages, we chose the most sociable flies (see detailed methods below). We ran two experimental sessions per day over 2 days, with each session including three male groups and three female groups from each of the eight lineages.

DETAILED ARTIFICIAL SELECTION METHODS

We housed selected flies for egg laying in groups of four males and four females in standard food vials with a sprinkle of live yeast for a total of 3 days, moving flies to a fresh set of vials with yeast after 2 days. We had 12 vials per lineage except for the parents of the first generation under selection, which we housed in population cages with food bottles. After egg laying, we transferred all the parental flies of a lineage (48 males and 48 females) to a single food bottle with live yeast for egg laying to generate a backup population for each lineage, kept at 18°C. We stored all egg-laying and housing vials and bottles in an environmental chamber at 25°C and 50% relative humidity (RH), and with a 12:12 light:dark cycle with lights on at 0900h.

Eleven days after egg laying, we collected newly eclosed virgin flies to be selected for the next generation. Within 8 h of

eclosion, we sexed 192 males and 192 females per lineage with light CO_2 anesthesia. We housed 16 same-sex flies in standard food vials for 96 h and checked on the test day that the females were virgin.

We performed the sociability selection assay in a room kept at 25°C and 50% RH. We ran four sessions of sociability testing and selection over 2 days to select 48 males and 48 females per selection lineage to produce the next generation (i.e., 25% truncation from 192 males and 192 females). At 0930h on the first day of testing, we added eight food discs to each of 48 arenas. At 1030h, we added flies to the arenas using gentle aspiration. We aspirated groups of 16 same-sex flies from the same holding vial at once into the central area of the arena by squeezing the aspirator between the foam and the plastic edge of the hole. From 1100h to 1230h, we allowed the flies to acclimatize. At 1230h, we blocked the central area of each arena by pushing down the foam plug, sealing the flies into the compartment that they had settled in. At this point, we recorded the number of flies in each compartment of each arena. We then selected flies to produce the next generation for each lineage based on the number of flies in each compartment. We removed flies by rotating the lid so that the off-center hole was above a particular compartment, then rotating the plastic door so that the hole was uncovered, and aspirating the flies out. For the low-sociability lineages, we selected four flies per arena from compartments with the lowest numbers of flies, unless those numbers were three or more, in which case we took flies from other replicate arenas of that session with smaller groups. Similarly, for the high-sociability lineages, we selected four flies per arena from the compartment(s) with the highest number of flies, unless that number was three or less, in which case we took flies from larger groups in replicate arenas. The unselected flies from each arena were discarded. After each of the four selection sessions, we ended with 12 males and 12 females selected per lineage. We then placed the selected flies in sex-specific holding vials.

At 1400h, we added flies for the second session to the same 48 dishes, recorded, collected selected flies at 1600h, and placed flies in single-sex vials. After the second session, we discarded the food discs and washed the arenas with 10% ethanol, allowing them to dry overnight. The following day, we ran sessions 3 and 4 in the same way. After all four sessions were completed at the end of test day 2, we mixed all selected flies within each lineage in a population cage to ensure among-vial gene flow, and then redistributed four males and four females into fresh food vials with a sprinkle of live yeast for egg laying.

To reduce the effects of genetic drift, we allowed for some flies to "migrate" between corresponding low and high lineages, similar to the strategy used by Turner and Miller (2012). Every other generation between generations 2 and 10, we selected two males and two females from each lineage to be transferred to a lineage selected in the opposite direction (i.e., on generation 2, Low sociability 1 to High sociability 1 and vice versa for each set of lineages; the paired lineages rotated on subsequent migration generations). We selected these flies based on the criteria for the lineage that they were "migrating" to. For example, for flies migrating from a high-sociability lineage to a low-sociability lineage, we selected flies that were alone in a compartment, or with the fewest number of other flies. We selected on sociability for 25 generations. Subsequently, we quantified the effect of 10 generations of relaxed selection. This period coincided with laboratory restrictions owing to the COVID-19 pandemic.

QUANTIFYING SOCIABILITY

Every generation, observers blind to selection treatment identity quantified a sociability score for each arena just after we lowered the foam plug using the formula: variance ÷ mean number of flies in each compartment (Scott et al. 2018). A sociability score of 0 indicated uniform fly distribution (two flies per compartment), a score of 1 implied random distribution, and any score significantly above 1 indicated significant sociability. A theoretical maximum sociability score of 16 could be achieved if all flies formed a single group within one compartment.

We also performed behavioral observations on a subset of arenas immediately after adding flies in generations 9 and 12. We intended to use these observations to gain insight into the interactions among flies at the beginning of the acclimatization period, as sociability scoring took place once these interactions had presumably occurred, and flies had settled in their preferred social arrangement among the compartments. In generation 9, we scanned 16 arenas in each of the 1230h and 1600h sessions for 1 min across three consecutive observation rounds, and in generation 12, we scanned 16 arenas in the morning session in the same way. The observer was blind to selection treatment identity, and the subset of arenas chosen included an equal number of arenas from each sex, treatment, and lineage. The only interactions we observed included low-level aggression (lunging in males, headbutting in females, and fencing in both sexes; Chen et al. 2002; Nilsen et al. 2004) and wing waving, which males use to signal to other males to back off (Paillette et al. 1991). These observations indicated that flies were mostly settled and showed very little movement within and between compartments after about 30 min into the acclimatization period.

ARTIFICIAL SELECTION DATA ANALYSIS

We analyzed generations 1–25 of the artificial selection experiment in a single mixed-effects general linear model, fitted using the lmer function from the R (version 4.0.4; R Core Team 2021) package lme4 (version 1.1-26; Bates et al. 2015). We took the $\log_{10}$ of the sociability scores as the response variable. This transformation allowed us to use a general linear model

without violating the assumption of normality of the residuals. Sex, generation, treatment, all their two- and three-way interactions, and test session (i.e., 1230h or 1600h observations) were fitted as fixed effects. Both the random intercept of test arena (which corresponds to the location the arena was placed in the test room) and random effects for the intercept and generation varying by lineage nested within treatment; however, the random slope included in the latter term was removed to reduce complexity in the random effects to fix a singular fit. Model assumptions of normality and homoscedasticity of the residuals were verified by inspecting plots of the results of the simulateResiduals function in the DHARMa package (version 0.3.3.0; Hartig 2020). Significance of the fixed effects was assessed using the Anova function from the car package (version 3.0-10; Fox and Weisberg 2019), and results of these tests are reported as Wald χ^2 test statistics and associated *P*-values.

We analyzed the effect of relaxed selection by fitting a model of sociability scores from generation 25, which was the last generation with artificial selection, and generation 35. The model was fitted and fixed effects tested in the same form as described above for the Generation 1–25 model, except with no three-way interaction in the fixed effects, and arena was fitted as a fixed effect instead of a random effect due to model convergence issues.

We analyzed the direct observations of aggressive and social interactions conducted in generations 9 and 12 as the presence or absence of behavior during the 1-min observation periods, using generalized linear mixed-effects models with a binomial distribution, fitted using the glmmTMB function in the glmmTMB package (version 1.0.2.1; Brooks et al. 2017). We modeled male and female low-level aggression separately as observations where aggression was present or absent as a function of treatment, generation, observation round, test session (1230h or 1600h), and test arena as fixed effects. We included the random effect of lineage nested within selection treatment. We modeled male social interactions (wing waving) using a separate model specified as above. Significance of the fixed affects was assessed as above.

EXPERIMENTS ON THE EVOLVED LINEAGES

Mating success and choice of males and females

In generation 28, we performed three experiments to assess mating success of flies from the low- and high-sociability selection lineages: male mating success with wild females, wild female mate choice between low- and high-sociability males, and wild male mate choice between low- and high-sociability females.

Male mating success (forced choice)

We measured the frequency of successful matings of individual males from the low- and high-sociability selection lineages paired with single females from a control population. Four days before testing, we sexed virgin males from the eight low and high selection lineages within 8 h of eclosion and housed them as in the regular selection procedure: 16 individuals per standard food vial. Two days before testing, we sexed virgin females from our standard wild population within 8 h of eclosion and housed them in vials of ∼10 individuals. We used 2-day-old females because our previous unpublished data indicated that such young females are reluctant to mate, with only 64% mating within 1 h. Starting at 0830h on test day, we added one male and one female to each empty test vial, and recorded whether a mating occurred within 1 h. We tested 40 males per lineage for a total of 320 males.

We analyzed the data with a generalized linear mixed-effects model with a binomial distribution using glmer from the lme4 package, and verified that the model assumptions were not violated with the DHARMa package. We modeled whether the male mated or not as a function of treatment, session, and the random effect of lineage nested within treatment, and tested the fixed effects with the Anova function.

Mate choice under competitive conditions in females and males

In the female mate choice experiment, we measured the mating frequency of males from the low- and high-sociability selection lineages with single control females when these females were given the choice between one low and one high sociable male. Such apparent mate choice, however, may be determined by male-male interactions including courtship interference (Baxter et al. 2018). Test males and females were reared and housed as with the male mating success protocol described above. One day before testing, half of the males were dusted with a pink, fluorescent powder to allow for identification during the test. Coloring was counterbalanced among selection treatments and lineages. Starting at 0830h on test day, using new empty vials, we added one uncolored male, then one pink male, and then the female. Observers blind to fly treatment recorded matings that occurred, and with which male, within 1 h of the trial start. We performed 70 trials for each of four Low- versus High-sociability competitions (i.e., males from each lineage competed against males from one other lineage of the opposite treatment: Low1 vs. High1, Low2 vs. High2, Low3 vs. High3, Low4 vs. High4), for a total of 280 trials.

In the male mate choice experiment, males from the control population were given the choice between one female from a low-sociability lineage and one female from a high-sociability lineage. The protocol for this experiment was similar to the female choice version, with the sexes reversed. We performed 70 trials for each of the same four High- versus Low-sociability competitions for a total of 280 trials.

To analyze the data from each of the two mate choice experiments, we modeled only the trials with successful matings against a 50:50 expectation for the two treatments. To do this,

we modeled the outcome of each competition using a binomial generalized linear mixed model with glmmTMB, with the combination of lineages competing as a random effect, test session and fly color as fixed effects, and tested for the model intercept being different from zero, which corresponds to a 50:50 mating success outcome for the two treatments on the logit scale.

Male-male aggression

We tested male-male aggression in flies from the low- and high-sociability selection treatments in generation 28 using our established protocol (Baxter and Dukas 2017). We sexed virgin males from the selection lineages within 8 h of eclosion, and housed them in standard food vials in groups of 16 for 96 h, as in the artificial selection protocol. Aggression arenas were 35-mm wide $\times$ 8-mm tall Petri dishes coated with Surfasil on the walls and underside of the lid to keep flies from walking on these areas. We covered the floor of each dish with a piece of circular filter paper, and placed a food patch (8-mm wide $\times$ 1.5-mm thick) topped with a 3-mm ball of thick yeast paste (5 g live yeast in 10 mL grapefruit juice) in the center.

At 0830h on the test day, we aspirated two males from the same lineage into each arena, and placed two arenas under each of six tripod-mounted Logitech c920 webcams, and recorded for 15 min. We repeated this for four consecutive recording sessions per day over 2 days, for a total of 96 trials (12 per lineage, 48 trials each per high and low selection treatments). We had one arena with high-sociability males and one with low-sociability males under each camera, and counterbalanced locations across sessions.

Observers blind to fly selection treatment recorded aggression behaviors via BORIS behavior observation software (version 7.9.8; Friard and Gamba 2016). We recorded the durations of the following aggressive behaviors to obtain a total duration of aggression for each trial: holding, lunging, boxing, and tussling (Chen et al. 2002; Baxter and Dukas 2017). We also recorded nonphysical aggressive displays in the form of wing threat.

We analyzed the data using a generalized linear mixed effects model with a Tweedie distribution and log link function, using glmmTMB. The Tweedie distribution is ideal for aggression data, which usually have a substantial mass at zero and positive skew (Dunn and Smyth 2005). We modeled the total duration of aggression in each trial as the response variable, selection treatment and test day as fixed effects, and observer, test session, arena, and lineage nested within treatment as random effects. We fit a separate model the same way to look at nonphysical wing threat. We verified that the assumptions of the models were not violated as before with the DHARMa package, and tested the fixed effects with Anova from the car package.

Female-female aggression

We also tested female-female aggression in two lineages from each of the low- and high-sociability selection treatments in generation 33. We sexed virgin females within 8 h of eclosion, and housed them in individual food vials for 96 h. We housed the females in isolation because female-female aggression is relatively rare, and isolation is known to increase aggression in females (Ueda and Kidokoro 2002). One day before testing, we added a male from our standard lab wild population (which was also derived from the same wild caught population as the selection lineages, and maintained in population cages of a few hundred individuals) to each female vial and observed for mating, which is also known to increase female aggression (Bath et al. 2017). After mating, we discarded the males. We used the same aggression arenas and test protocol as described in the male-male aggression experiment, except that videos were recorded for 20 min.

We performed 96 trials over 2 days (24 per lineage, 48 per low- and high-sociability treatment). An observer blind to fly selection treatment recorded aggression behaviors via BORIS software, including head-butting, lunging, and pushing (i.e., one female pushing another off the food disc with her front legs), to obtain a total duration of aggression.

We analyzed the female-female aggression data as with the male-male data, except without an observer random effect term as there was only one observer.

Alternative measure: Nearest neighbor distance

We tested male and female flies from the selection lineages in generation 28 for their level of social behavior as measured by the median NND of single-sex groups in a homogenous open arena (Anderson et al. 2016). We sexed flies within 8 h of eclosion, and housed them in same-sex groups of 14 for 72 h prior to testing. For test arenas, we used 35-mm Petri dishes with 8 mL of standard food (cornmeal omitted for video clarity with automated tracking) covering the bottom, effectively constraining the flies to two dimensions. At 0900h on the test day, we briefly anesthetized the flies with CO_2 and transferred 12 from the same vial into each arena. We allowed the flies 5 h to acclimatize. We then transferred the arenas in groups of 10 to each of six climate-controlled semitransparent test boxes equipped with overhead webcams. We allowed the flies an additional 30 min to acclimatize to the test boxes, at which point they were mostly settled, and then recorded the arenas for 30 min.

We performed two consecutive recording sessions of 60 arenas per day ($\sim$1500h-1530h and $\sim$1600h-1630h) over 2 days for a total of 120 arenas per sex (15 arenas per lineage, 60 per selection treatment). We used the same custom Python script to automate video analysis described in Anderson et al. (2016) that samples frames of video every 30 s and calculates the NND

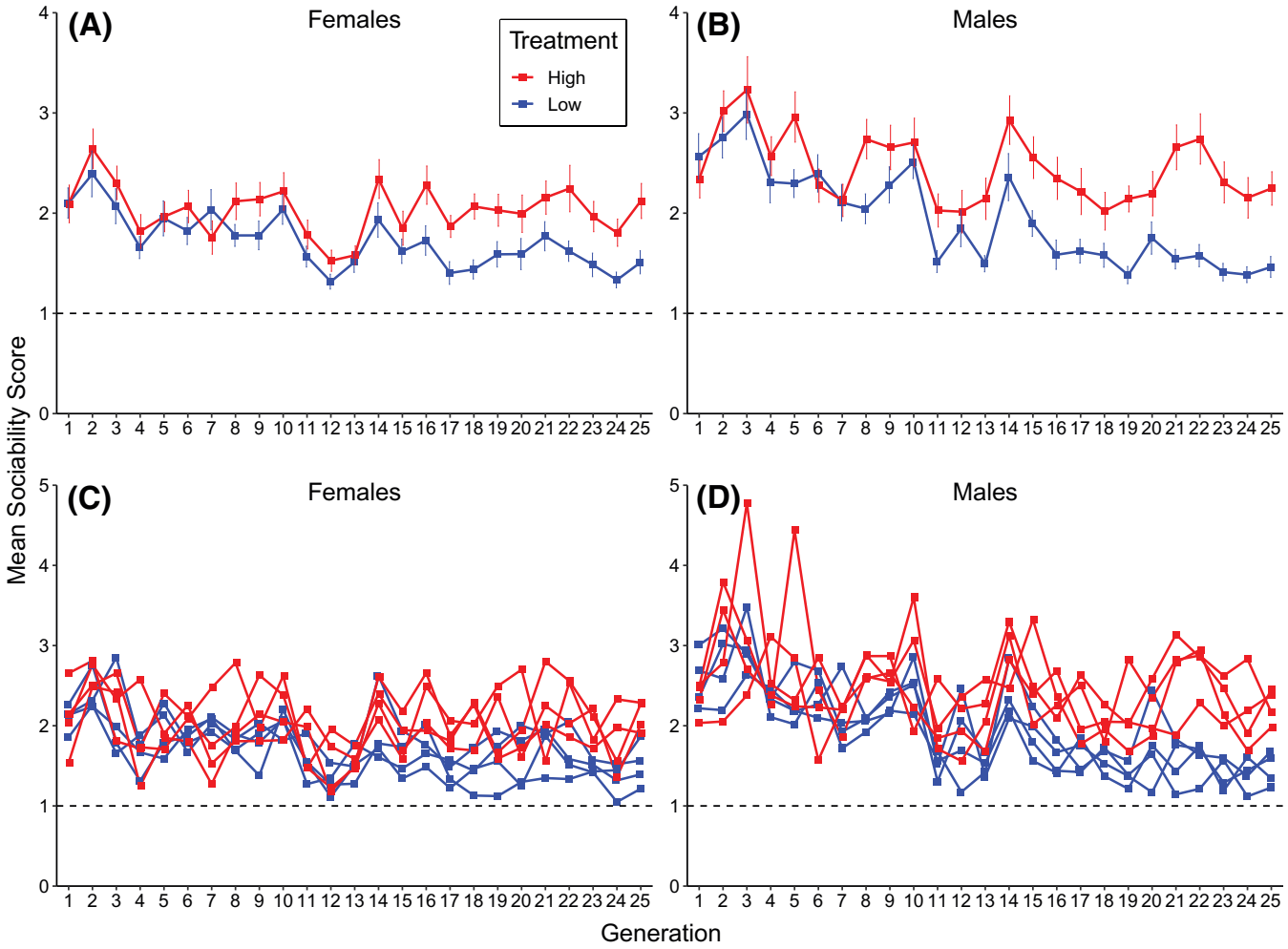

Figure 2. **Divergence in selection treatments in sociability score over 25 generations. Mean ± SEM sociability scores across all selection lineages for low- and high-sociability treatments in (A) females and (B) males. The same data are displayed by replicate lineages (error bars excluded for clarity) in (C) females and (D) males. Values significantly above 1 (dashed lines) indicate significant sociability.**

of each fly (i.e., for each fly, the distance between its centroid and the centroid of the closest fly), which was then used to calculate the median NND for each arena as a measure of sociability.

We analyzed the data with a general linear mixed effects model using the lmer function of the lme4 package, and verified model assumptions were not violated as before. We used the mean of the median NND of each arena across the duration of the trial to obtain one value for each trial to model as the response variable. We modeled test day, session, treatment, sex, and treatment × sex as fixed effects, and test box, arena, and lineage nested within treatment as random effects. We tested the significance of the fixed effects using the Anova function from the car library.

Results

SOCIABILITY ARTIFICIAL SELECTION

There was a significant effect of our artificial selection regime on the sociability of the low- and high-sociability treatments, with the lineages starting the experiment at the same sociability level and then diverging (Generation × Treatment interaction: $\chi^2_1 = 48.75$, $P < 0.001$; Fig. 2). Males were more sociable than females ($\chi^2_1 = 66.53$, $P < 0.001$; Fig. 2). By the end of the experiment, female flies from the high-sociability treatment had, on average, about a 40% higher sociability score compared to the low treatment, and males from the high treatment had about a 54% higher sociability score compared to the low treatment (Main effect of Treatment in Generation 25: $\chi^2_1 = 25.18$, $P < 0.001$; Fig. 2).

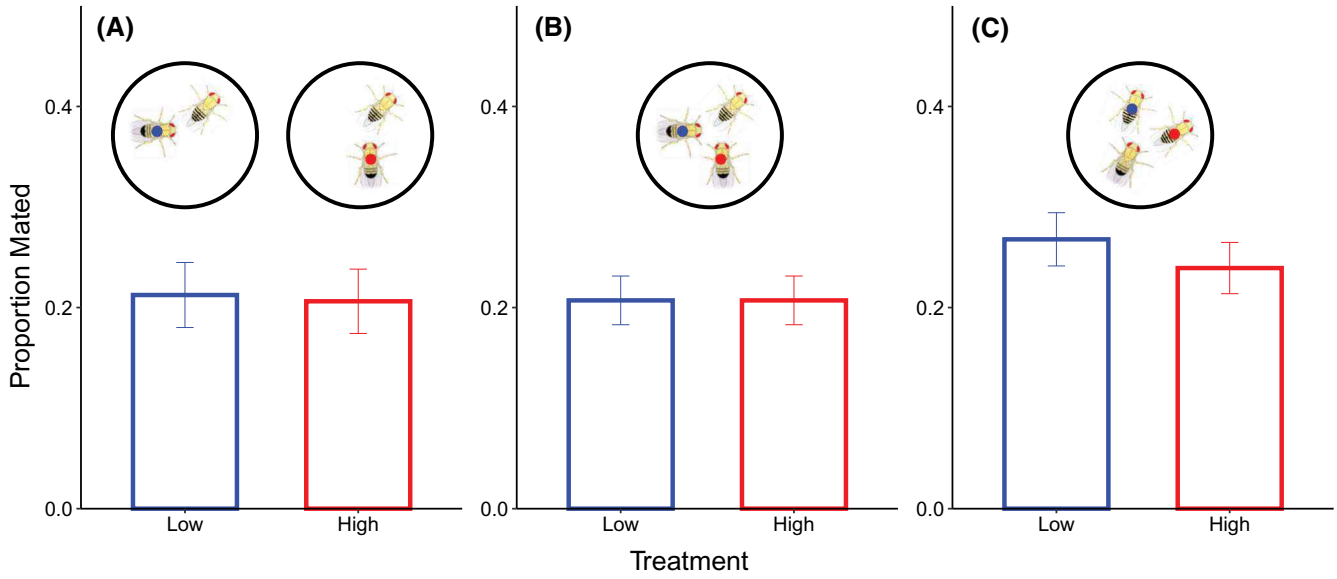

Figure 3. **Mating success of selected males and females. Males can be identified by the black tip of their abdomen. Flies from the low-sociability lineages are marked with blue dots, flies from the high-sociability lineages are marked with red dots, and flies from the control population are unmarked. (A) Mating success of single males from the selection treatments with single control females. The maximum possible value of each bar is 1. (B) Competitive mating success of males from the selection treatments in vials each containing a single control female and one low- and one high-sociability male. Here, the maximum possible value of both bars combined is 1. (C) competitive mating success of females from the selection treatments in vials each containing a single control male and one low- and one high-sociability female. The maximum possible value of both bars together is 1. Colored dots on flies in the cartoons are only to distinguish treatments in this figure, and were not applied in the actual experiment. Error bars show ± the standard error of the proportion p, $\sqrt{p(1-p)/n}$. The 95% confidence intervals for the nonsignificant treatment effects are (A) [−0.27, 0.31], (B) [−0.34, 0.30], and (C) [−0.28, 0.46].**

In our behavioral observations of a subset of arenas in generations 9 and 12, we only recorded a few cases of low-level aggression in a small proportion of the arenas, which occurred at similar frequencies in the low and high lineages (proportion of arenas with aggression, females: low sociability = 0.31, high sociability = 0.19; $\chi^2_1 = 1.78$, $P = 0.18$; males: low sociability = 0.11, high sociability = 0.17; $\chi^2_1 = 0.44$, $P = 0.51$). We also recorded a few cases of social interactions in the form of wing waving among males, which were also not significantly different among selection treatments (proportion of arenas with social interactions, low sociability = 0.25, high sociability = 0.36; $\chi^2_1 = 0.78$, $P = 0.38$).

MATING SUCCESS

We did not detect a significant effect of selection treatment on individual male mating success with single control females ($\chi^2_1 = 0.020$, $P = 0.89$; Fig. 3A), on male mating frequency with single control females given a choice between one low- and one high-sociability male ($z = 0.10$, $P = 0.92$; Fig. 3B), or on female mating frequency with single control males given the choice between one low- and one high-sociability female ($z = -0.39$, $P = 0.70$; Fig. 3C).

FEMALE-FEMALE AND MALE-MALE AGGRESSION

Low-sociability females were significantly more aggressive than high-sociability females ($\chi^2_1 = 12.20$, $P < 0.001$; Fig. 4A). Similarly, low-sociability males were significantly more aggressive than high-sociability males ($\chi^2_1 = 4.05$, $P = 0.044$; Fig. 4B). We did not, however, observe a significant difference in time spent performing wing threat between selection treatments (mean ± SEM, low sociability = 0.35 ± 0.18 s/min; high sociability = 0.39 ± 0.12 s/min; $\chi^2_1 = 0.005$, $P = 0.95$).

RELAXED SELECTION

We did not observe a significant effect of 10 generations of relaxed selection after stopping selection with generation 25 (Generation × Treatment interaction: $\chi^2_1 = 1.02$, $P = 0.31$; Fig. 5). In generation 35, the significant effect of selection treatment remained ($\chi^2_1 = 30.25$, $P < 0.001$; Fig. 5).

ALTERNATIVE MEASURE: NEAREST NEIGHBOR DISTANCE

We did not detect a significant main effect of selection treatment on NND ($\chi^2_1 = 0.06$, $P = 0.81$; Fig. 6). Overall, males had smaller NNDs than females ($\chi^2_1 = 19.22$, $P < 0.001$; Fig. 6), and the

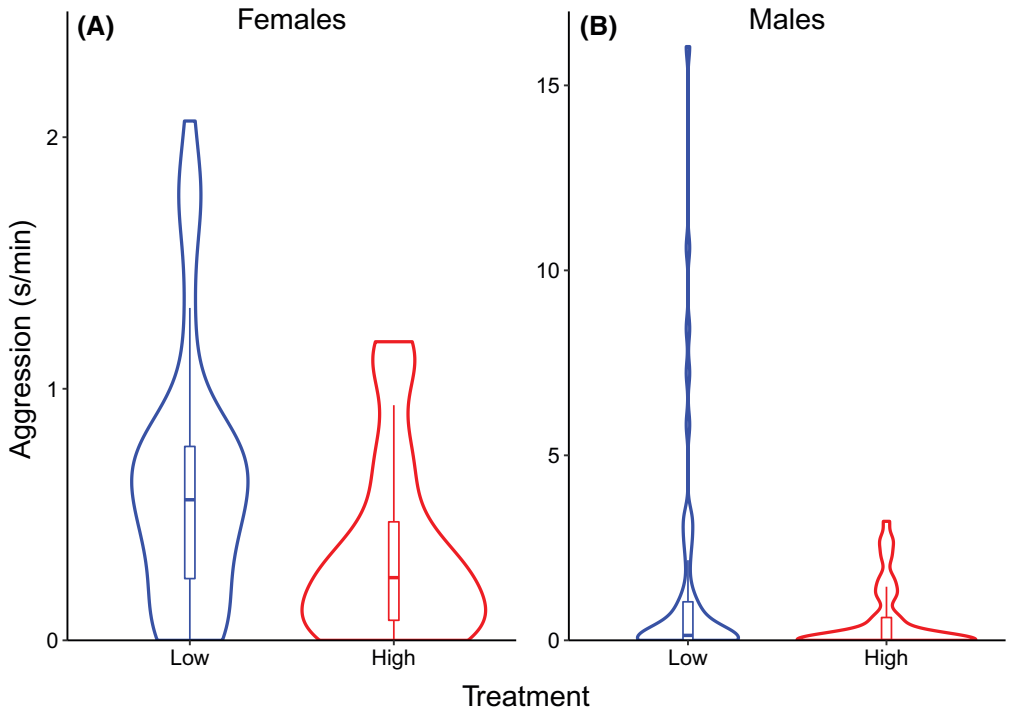

Figure 4. Aggression frequency in (A) females and (B) males from the selection treatments after 25 generations of selection. Inner box plots show median, interquartile range (IQR), and whiskers up to 1.5 × IQR. Outer violin plots show the shape of the distribution of the data.

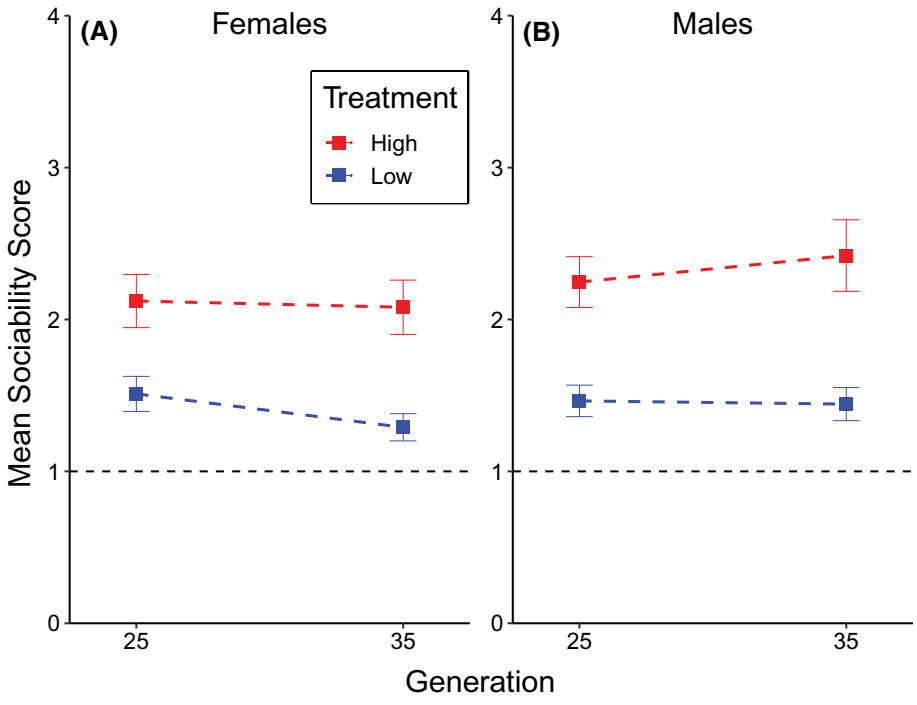

Figure 5. Mean ± SEM sociability scores at the end of 25 generations of selection, and after 10 generations of relaxed selection in (A) females and (B) males. Values significantly above 1 (dashed lines) indicate significant sociability.

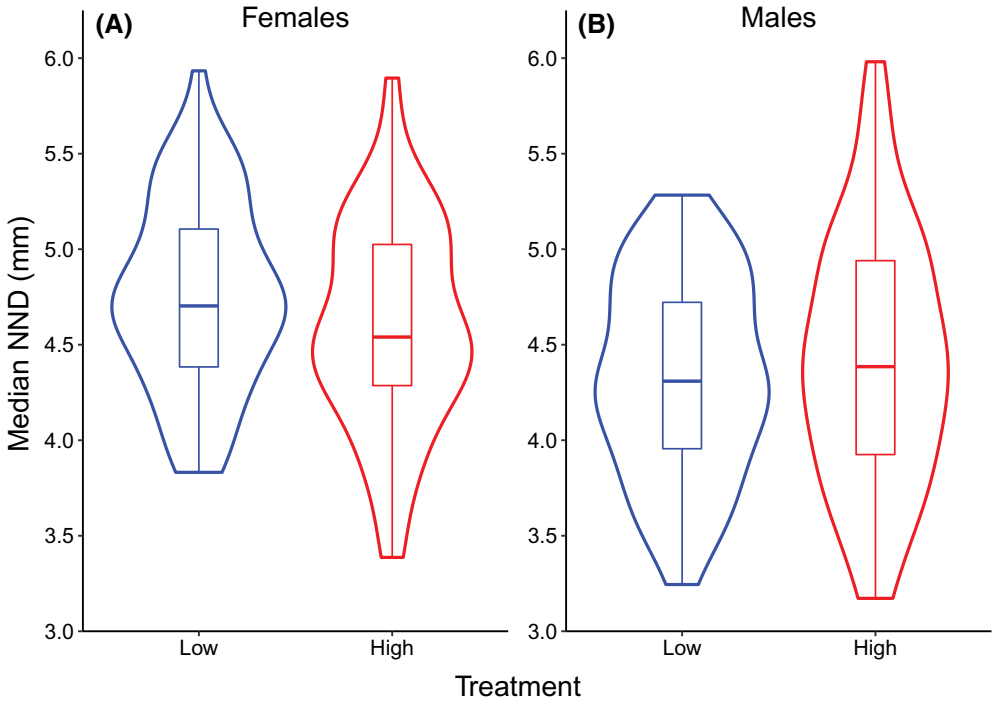

Figure 6. The median nearest neighbor distances in (A) females and (B) males, after 25 generations of selection. Inner box plots show median, interquartile range (IQR), and whiskers down and up to 1.5 × IQR. Outer violin plots depict the data distribution. The 95% confidence interval for the nonsignificant treatment effect is [−0.12, 0.15].

treatment-by-sex interaction approached significance ($\chi^2_1 = 3.28$, $P = 0.070$; Fig. 6).

Discussion

Our key findings were first, that we were able to generate significant divergence in sociability scores between the selection treatments over 25 generations of artificial selection in both females and males (Fig. 2). This resulted in relatively 40% higher sociability scores in high-sociability females, and relatively 54% higher sociability scores in high-sociability males. Second, flies from the low and high lineages had similar mating success (Fig. 3). Third, low-sociability females and males had higher levels of intrasexual aggression compared to their high-sociability counterparts (Fig. 4). Fourth, the low- and high-sociability lineages did not converge even after 10 generations of relaxed selection (Fig. 5). Finally, the low- and high-sociability lineages did not differ in their NND (Fig. 6). We will discuss each of these findings in turn.

By successfully evolving via artificial selection lineages of low and high sociability in a highly tractable model system, we pave the way for further investigations on the ecology and evolution of a central phenotypic trait that structures behavior and determines fitness in numerous species including humans. For example, long-term field observations on savanna and chacma baboons (*Papio cynocephalus* and *Papio hamadryas ursinus*) indicated that females with stronger and more stable social bonds lived longer and had higher infant survival rates (Silk et al. 2003, 2010). In another well-studied system, many species of fish move in tight groups typically referred to as schools. Field observations, which were followed up by controlled laboratory studies, indicated that Trinidad guppies (*Poecilia reticulata*) from distinct populations that vary in predation risk show heritable variation in school size, with guppies from high predation pools having larger and more cohesive groups as well as higher survival rates when exposed to predators (Seghers 1974; Magurran et al. 1992; O'Steen et al. 2002; Huizinga et al. 2009). Recently, Kotrschal et al. (2020) artificially selected for three generations on guppies' group polarization, which is the tendency of school members to align with each other's directional movement. This led to significant increases in polarization and cohesiveness in females. Finally, humans show heritable variation in sociability and there is a strong positive correlation between the quality of social relationships and both health and life expectancy (House et al. 1988; Holt-Lunstad et al. 2010; Day et al. 2018; Abdellaoui et al. 2019).

Although high levels of sociability positively affect fitness in some species, they could have negative effects in others. For this reason, we predicted that our evolved high-sociability lineages would show some decrements in performance. Specifically, we expected sociable males to have lower mating success because we

assumed that they might be less aggressive in pursuing reluctant females. However, we found no differences in mating success between males from the low and high lineages under both no choice and choice experiments (Fig. 3). Similarly, females from the low and high lineages had similar mating success (Fig. 3). Apparently, selection on sociability affects neither courtship behavior nor attractiveness to the other sex.

Unlike the sexual features, selection on sociability led to a correlated change in aggression (Fig. 4). One can then argue that, although we quantified sociability, we actually selected on aggression. We should note, however, that our direct observations on flies just after we set up the sociability arenas during the artificial selection stage indicated low frequencies of only low-level aggression. This was not surprising because we housed all flies in groups of 16 same-sex individuals from sexing through testing, and such group settings are associated with low levels of aggression (Wang et al. 2008). Furthermore, in an earlier work quantifying genetic variation in sociability, we found that genotypes that varied widely in sociability did not show significant variation in aggression (Scott et al. 2018). Nevertheless, our current results suggest a negative correlation between sociability and aggression, which we intend to explore further in our ongoing genomic work on the evolved sociability lineages.

One may argue that it is obvious that flies that prefer to be in groups would be less aggressive. Following this logic, we also expected that sociable flies would show shorter NND when tested in small arenas designed to quantify this alternative measure of social behavior (Simon et al. 2012; Anderson et al. 2017). Surprisingly, however, our low- and high-sociability lineages did not differ in their NNDs (Fig. 6). This result illustrates that social behavior is a complex trait and that apparently related social features may have distinct genetic bases. Somehow the cues, signals, and mechanisms that determine individuals' tendency to form groups differ from the ones that affect NNDs. That is, regardless of individuals' tendencies to seek and tolerate others at the same food patch, they seem to have a similar preferred minimum interindividual distance when compelled to share a single patch. Although it sounds counterintuitive, interindividual distance has been well studied in a variety of social animals, in which individuals simultaneously balance their social attraction to as well as minimum distance from others (Hall 1966; Sorokowska et al. 2017). For example, in black headed gulls (*Larus ridibundus*), members of the flock maintain distance through a combination of avoidance and mild threat (Conder 1949). Our recent genetic work indeed indicates distinct genetic effects on NND and sociability (figs. 2A, B vs. figs. 4A, B in Yost et al. 2020), and we intend to further characterize the sociability phenotype in our ongoing genomics work.

Although we measured a few parameters in the evolved lineages, there may have been other correlated traits that have changed with sociability. Because we selected on sociability scores lower and higher than the likely optimal sociability levels in the baseline population, we expected some fitness costs associated either with sociability or other correlated traits that would lead to convergence of the low and high lineages toward the initial sociability scores. Such convergence under relaxed selection is rather common. For example, artificial selection on phototaxis in *D. pseudoobscura* led to rapid divergence of the negative and positive selection lineages followed by quick convergence under relaxed selection (Dobzhansky and Spassky 1969). In our case, however, we found no convergence under relaxed selection (Fig. 5). Apparently, there are no costs associated with possessing below and above the sociability scores of wild fruit flies under the specific parameters of our protocol. Nevertheless, such costs may exist in both natural settings and population cages in the laboratory. For example, costs of high sociability could include increased larval competition if females lay more eggs on a portion of the available food patches (Atkinson 1979; Grimaldi and Jaenike 1984; Durisko and Dukas 2013; Golden and Dukas 2014). Our protocol, however, did not allow for this to happen because we collected eggs for the next generation only when flies were in low-density vials with ample live yeast and media.

Our current and previous works as well as research in other laboratories indicate that fruit flies have rich social life. Importantly, wild fruit flies spontaneously form social groups under controlled natural settings (Dukas 2020). They show heritable variation in sociability (Figs. 2 and 5; Scott et al. 2018) as well as related social traits (Wice and Saltz 2021). Fruit flies, however, also engage in aggressive encounters within their naturally occurring social groups (Dukas 2020) and show heritable variation in such aggression (Hoffmann and Cacoyianni 1989; Dierick and Greenspan 2006; Edwards et al. 2006). Fruit flies are socially influenced by each other (Levine et al. 2002), socially learn relevant information about egg-laying substrates (Sarin and Dukas 2009; Battesti et al. 2012), and their collective behavior enhances their responses to hazards (Ramdya et al. 2015; Ferreira and Moita 2020). We failed, however, to identify costs associated with the evolved sociability values, which were lower or higher than those in the initial wild population, and will keep pursuing this topic in future work.

Overall, we succeeded in generating via artificial selection fly lineages that show low and high sociability and to employ the evolved lineages for addressing relevant questions about the evolutionary biology of sociability. We found that variation in sociability is not associated with either attractiveness or competitive ability in a mating context, that sociability is genetically negatively correlated with intrasexual aggression, but that it is not positively correlated with flies' preferences for interindividual distance. Finally, there were no other costs to the evolved lower and higher levels of sociability as 10 generations of relaxed

selection did not lead to convergence of the selected low- and high-sociability lineages. As expected, sociability is a complex trait, which we will keep studying through our ongoing genomics and gene expression work on the evolved sociability lineages.

AUTHOR CONTRIBUTIONS

AMS, ID, and RD designed the experiments. AMS ran the artificial selection and follow-up experiments. RD did live behavioral observations in generations 9 and 12. AMS analyzed the data. AMS and RD wrote the first draft. All authors contributed to the revisions.

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DATA ARCHIVING

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.qrfj6q5gx.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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