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Selection on learning performance results in the correlated evolution of sexual dimorphism in life history

Martyna K. Zwoinska,[1,2] Martin I. Lind,[1] Maria Cortazar-Chinarro,[1] Mark Ramsden,[1] and Alexei A. Maklakov[1]

[1]*Department of Animal Ecology, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden*

[2]*E-mail: martyna.zwoinska@ebc.uu.se*

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The evolution of learning can be constrained by trade-offs. As male and female life histories often diverge, the relationship between learning and fitness may differ between the sexes. However, because sexes share much of their genome, intersexual genetic correlations can prevent males and females from reaching their sex-specific optima resulting in intralocus sexual conflict (IaSC). To investigate if IaSC constraints sex-specific evolution of learning, we selected *Caenorhabditis remanei* nematode females for increased or decreased olfactory learning performance and measured learning, life span (in mated and virgin worms), reproduction, and locomotory activity in both sexes. Males from downward-selected female lines had higher locomotory activity and longer virgin life span but sired fewer progeny than males from upward-selected female lines. In contrast, we found no effect of selection on female reproduction and downward-selected females showed higher locomotory activity but lived shorter as virgins than upward-selected females. Strikingly, selection on learning performance led to the reversal of sexual dimorphism in virgin life span. We thus show sex-specific trade-offs between learning, reproduction, and life span. Our results support the hypothesis that selection on learning performance can shape the evolution of sexually dimorphic life histories via sex-specific genetic correlations.

KEY WORDS: *Caenorhabditis*, **cognition, intralocus sexual conflict, olfactory learning, sex-specific life histories.**

Evolutionary biology of cognition is a relatively new field, and its important contribution has been to reveal the costs and benefits of cognitive traits, in particular of learning and memory (Dukas and Bernays 2000; Dukas and Duan 2000; Mery and Kawecki 2003, 2004, 2005; Burger et al. 2008; Snell-Rood et al. 2011; Cole et al. 2012; Cole and Quinn 2012; Lagasse et al. 2012; Placais and Preat 2013). The ability to learn can increase fitness and studies on insects found slower growth and reduced reproduction in animals prevented from learning compared to controls (Dukas and Bernays 2000; Dukas and Duan 2000). However, cognitive traits can be costly as illustrated by the studies showing that artificial selection on improved learning resulted in lower larval competitive ability and shorter life span in *Drosophila melanogaster* (Mery and Kawecki 2003; Burger et al. 2008; Lagasse et al. 2012). Similarly, full-sib families of the butterfly *Pieris rapae* with higher learning ability suffered from reproductive delay (Snell-Rood et al. 2011). Not only intrinsically better learning ability or memory entail fitness costs; learning experience and formation of long-term memory alone can also result in lower reproductive output (Mery and Kawecki 2004; Snell-Rood et al. 2011) and decreased stress resistance (Mery and Kawecki 2005; Placais and Preat 2013).

Males and females often differ in cognitive traits (Bachevalier and Hagger 1991; Shettleworth 2001; Jones et al. 2003; Lonsdorf et al. 2004; Jonasson 2005; Jozet-Alves et al. 2008; Dalla and Shors 2009; Guigueno et al. 2014; Miller and Halpern 2014). Given that cognitive traits can trade-off with life-history traits (see above) and that males and females often have different reproductive strategies (Bateman 1948; Trivers 1972; Kokko and Jennions 2008; Scharer et al. 2012; Maklakov and Lummaa 2013), the costs and benefits of cognition can differ between the sexes. In *Drosophila*, males from populations evolving under experimentally enforced monogamy performed worse in olfactory learning assays and courtship compared to polygamous males, suggesting a direct link between the strength of sexual selection and

EVOLUTION
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learning (Hollis and Kawecki 2014). Similarly, in *Caenorhabditis* nematodes mating poses greater sensory and motor challenges for males than for females (Barr and Garcia 2006). Importantly, sexual selection is not the only force that may select for improved aspects of cognition in a sex-specific manner. For instance, female cowbirds have better spatial cognition than males, which is linked to brood parasitism in this species—only females search for hosts nests (Guigueno et al. 2014). Yet, despite theoretical expectations and emerging empirical evidence, only a limited number of studies examined sex-specific trade-offs between cognitive and life-history traits (Burger et al. 2008; Cole et al. 2012; Lagasse et al. 2012; Zwoinska et al. 2013; Carazo et al. 2014; Hollis and Kawecki 2014; Ostojic et al. 2014; Kotrschal et al. 2015).

Importantly, sexual dimorphism in cognitive traits—a historical signature of sex-specific selection, does not imply that the sexes are at their optimum for a particular trait (Harano et al. 2010) or their life histories as a whole (Lewis et al. 2011; Berger et al. 2014). Intersexual genetic correlation can constrain independent evolution of the sexes (Lande 1980; Rice 1984; Chippindale et al. 2001; Bonduriansky and Chenoweth 2009) resulting in multivariate intralocus sexual conflict (IaSC) (Prasad et al. 2007; Kwan et al. 2008; Abbott et al. 2010; Lewis et al. 2011; Gosden et al. 2012; Berger et al. 2014). Suits of genetically integrated life-history and behavioral traits are predicted to be hotspots of sexually antagonistic genetic variation (Mank et al. 2008; Bonduriansky and Chenoweth 2009) aggravating IaSC over individual traits (Berger et al. 2014). The resolution of multivariate IaSC requires sex-specific genetic covariances, but their evolution is expected to be slow (Lande 1980, 1981; Harano et al. 2010). Recent studies in insects provide support for the existence of multivariate IaSC over life-history syndromes (Abbott et al. 2010; Bedhomme et al. 2011; Lewis et al. 2011; Delcourt et al. 2012; Berger et al. 2014; Gosden and Chenoweth 2014). However, other studies also report high levels of sex-specific genetic variation for such important and potentially widely pleiotropic life-history trait as life span (Lehtovaara et al. 2013; Chen and Maklakov 2014). Therefore, whether sex-specific evolution of cognitive performance is constrained by genetic covariances resulting in IaSC is an empirical question and we need more studies on life-history syndromes in different taxa to advance our understanding of the relative importance of multivariate IaSC in the evolution of cognition. The sexually dimorphic olfactory learning performance in the dioecious nematode worm *C. remanei* (Zwoinska et al. 2013) offers such opportunity.

In this study, we investigated sex-specific fitness effects of selection on learning performance in an olfactory learning task. We used the nematode worm *C. remanei*, which exhibits sex-specific learning performance (Zwoinska et al. 2013). Locating females requires detecting female sex pheromone, which in turn requires male-specific cephalic neurons (Chasnov et al. 2007). Given the importance of olfaction in mate search (and possibly in other stages of mating sequence) in *C. remanei* males, it is likely that reduced olfactory learning performance will translate into reduced mating performance, and therefore reduced fitness. We employed a well-established olfactory learning protocol based on making an association between a food and an odorant—butanone (Torayama et al. 2007; Kauffman et al. 2010; Kauffman et al. 2011; Zwoinska et al. 2013). To aid the investigation of IaSC, we selected on one sex only—females, as they are easier to handle than males. After 13 generations of selection on improved and decreased female performance in the olfactory learning task, followed by four generations of relaxed selection, we measured age-specific learning performance in both sexes at days 2 and 7 of adulthood (i.e., during peak reproduction and after the reproductive peak but before the onset of mortality). To investigate the effect of selection of the correlated evolution of sex-specific life histories, we also measured sex-specific life span of virgin and reproducing worms, locomotory activity, and reproductive performance. We predicted that (1) selection on females will affect both sexes because of an intersexual genetic correlation for learning performance; and (2) reduced learning performance will reduce male fitness. More broadly, we wanted to test whether evolution of learning performance is constrained by between-trait between-sex genetic correlations (i.e., by multivariate IaSC) or whether sex-specific genetic architecture will allow for relatively independent correlated evolution of life-history, behavioral, and cognitive traits in males and females.

Materials and Methods

WORM STRAIN AND CULTURE

We used the SP8 strain of *C. remanei*, provided by N. Timmermeyer from the Department of Biology at Tuebingen University, Germany (Fritzsche et al. 2014). This strain harbors substantial amount of genetic variation for fitness and life-history traits (Chen and Maklakov 2012). Cryopreserved worms were unfrozen and propagated for two generations prior to the start of the experiments. Throughout the experiments, we maintained our populations on 92 mm Petri plates poured with NGM agar medium and seeded with 1 ml of *Escherichia coli* as food (Stiernagle 2006). Plates with animals were kept in darkness in climate chambers set at 20°C and 60% humidity. Hypochlorite treatment (bleaching) was used to obtain age-synchronized populations, as it leaves only eggs, killing larvae and adults (Stiernagle 2006). Under standard conditions *C. remanei* eggs hatch about 12–20 h after being laid with a peak 14 h after egg deposition (Diaz et al. 2008). In fitness and locomotory activity assays the antibiotics streptomycin, kanamycin, and nystatin were added to the agar and LB (Luria Bertani) bacterial medium to prevent infections (Lionaki and Tavernakis 2013). In these experiments, we used the antibiotic

resistant *E. coli* OP50 1 (pUC4K) provided by J. Ewbank from the Centre d'Immunologie de Marseille-Luminy. For selection and all other experiments, we used the standard *E. coli* OP50.

GENERAL SELECTION PROCEDURE

Every generation young, day 2 of adulthood, females were selected for performance in an olfactory learning task. After the selection procedure, 200 gravid females were collected to establish a new generation (see Fig. S1). We conducted 13 generations of selection. The progeny of 200 females from the previous generation were kept together until day 2 of adulthood when selection was applied. By this time, females were multiply mated with males from the entire population. Although both sexes went through the learning assays only females were retained and the next generations of females were also mated multiply with all males in their population before selection was applied. This protocol allowed for selection on learning in females but also allowed for correlated evolution in males, which would not be possible if we would have mated females from each line to ancestral males.

Our experimental rationale was based on exposing one sex to directional sex-specific selection on the trait of interest (here, olfactory learning performance) and testing for the evolutionary response to selection and fitness consequences of such a response in both sexes. We used females for logistic reasons because male mate search behavior in *Caenorhabditis* nematodes causes them to wander off the bacterial lawn, often resulting in accidental deaths. Each treatment consisted of three replicates: three upward-selected and three downward-selected lines. One upward line and one downward line were always selected on the same day and form an experimental block. At the end of the selection experiment, the worms from each selection regime were allowed to reproduce for two more generations and then cryopreserved at −80°C (Stiernagle 2006). The populations also underwent two generations without selection after they were thawed, before the start of the learning, life span, fitness, and activity assays. Thus, the evolutionary response to selection was measured after four generations of relaxed selection, which greatly reduces the possibility for nongenetic effects to affect the results. The ancestral populations were also cryopreserved in the beginning of the selection and their learning performance was tested together with the upward- and downward-selected lines.

SELECTION LEARNING ASSAY

The learning task employed during selection measured the ability of the worms to change their naive preference for benzaldehyde to a preference for butanone by associating butanone with bacterial food. Both butanone and benzaldehyde are bacterial metabolites and attractants for *Caenorhabditis* nematodes. They are well studied in the context of chemoattraction, associative and nonassociative learning (Colbert and Bargmann 1995; Nuttley et al. 2002; Torayama et al. 2007; Tsui and van der Kooy 2008; Kauffman et al. 2010; Kauffman et al. 2011; Zwoinska et al. 2013). In *C. elegans*, a single sensory neuron (AWC) is known to respond to these odorants, and evidence suggests that it may also play a role in other steps of butanone olfactory learning (Troemel and Kimmel 1997; Torayama et al. 2007). Worms that retained a preference for benzaldehyde after the learning task were considered nonlearners (downward selection), whereas worms that switched their preference to butanone were considered learners (upward selection) (Fig. S1).

The selection assay constituted of a modified version of the butanone olfactory learning protocol originally developed for *C. elegans* (Torayama et al. 2007; Kauffman et al. 2010; Kauffman et al. 2011), and then successfully applied to *C. remanei* (Zwoinska et al. 2013). The learning step—simultaneous presentation of food (*E. coli*) and butanone to worms follows the original protocol (Kauffman et al. 2011). However, in the modified version of the assay we assessed postconditioning response in the presence of both butanone and benzaldehyde and not ethanol (solvent), as in the original protocol (Fig. S2). Therefore, the modified protocol was based on a change in preference from the highly attractive benzaldehyde to butanone, by associating butanone with food. This constitutes a stronger test of learning than to simply score increased attraction to butanone.

During selection, day 2 old adult worms of both sexes—the progeny of 200 mated females from the previous round of selection (or from the ancestral population for the first round) were suspended in M9 buffer (Stiernagle 2006) and then pipetted onto the center of the 150 mm assay Petri plate (which contained NGM agar but no food) (Fig. S2). Then, 1 μl of 10% butanone was pipetted on one side of the plate and 1 μl of 30% benzaldehyde on the other side of the plate. Next, the remaining M9 buffer was removed with a corner of a Kim Wipe cleaning tissue and the worms were left for one hour. After this time, only the worms from the benzaldehyde side (i.e., showing high naive attraction to benzaldehyde) were collected for further testing (naive worms prefer benzaldehyde over butanone). The aim of this step was to remove worms with preference for butanone in both upward- and downward-selection treatments. In the second step, the worms that showed initial attraction to benzaldehyde were conditioned in the presence of butanone. The worms were left for one hour on conditioning plates containing food—500 μl of *E. coli* OP50 and 2 μl of butanone streaked on the lid of the plate. With such amount of food the nematodes crawl in bacterial slurry, assuring ad libitum food availability. During this time, the worms associated the smell of butanone with food. In the third step, performed directly after conditioning, the worms were once again placed on 150 mm assay plates and again presented with 1 μl of butanone and 1 μl of benzaldehyde originating from either side of the plate and left for one hour. For the upward-selected lines, only

females from the butanone side of the plate were collected, and for downward-selected lines only the females from the benzaldehyde side were collected (worms that failed to leave the place of origin were disregarded). The distance to cover and the time given to reach each odorant spot was the same for both selection regimes, assuring no direct selection on locomotory activity. The assays were always run in replicate runs. After each assay, 200 females from replicate population were collected from the odorant spot and allowed to lay eggs, which were isolated by hypochlorite treatment the next day. Once the eggs developed into young adults, the progeny of the selected females were allowed to mate with each other within each replicate population and were used during subsequent rounds of selection when reaching day 2 of adulthood.

POSTSELECTION LEARNING ASSAYS

The postselection learning assays were performed to investigate the response to selection. We employed two learning protocols in two separate experiments: first, the selection protocol as described above (henceforth, Experiment 1) and second, the butanone olfactory learning protocol originally developed for *C. elegans* (Kauffman et al. 2010, 2011), henceforth, Experiment 2 (Fig. S2). Although the conditioning step was the same for both experiments, naive and postconditioning chemotaxis to butanone was measured two ways: (1) in the presence of benzaldehyde (strong attractant) or (2) in the presence of ethanol (solvent). Because benzaldehyde was used during the selection procedure and we found that the lines differed in their response to benzaldehyde when butanone was present, we wanted to demonstrate that the association between butanone and food was still formed even in the absence of benzaldehyde, and therefore that the differences in learning performance observed between our lines was still present. To collect data on sex-specific learning performance after selection, males and females were scored separately. The day before the learning assays, 125 females and 150 males (day 1 of adulthood, the worms were already mated at the time of collection) were separated on 60 mm plates with 500 μl of food. In Experiment 2 we used 92 mm plate (150 mm in Experiment 1; see Fig. S2 for the design of assay plates). We used the larger 150 mm plates in Experiment 1, so that the setup mirrored the selection procedure, whereas in Experiment 2 we followed the original butanone olfactory learning protocol (Kauffman et al. 2010, 2011). However, in contrast to the Kauffman protocol, we did not employ one hour of starvation before the conditioning in neither of our two experiments. In fact, butanone olfactory learning was first demonstrated without the period of food deprivation later introduced by Kauffman (Torayama et al. 2007). Additional details of the butanone olfactory learning protocol are described elsewhere and a demonstrational video is available at http://www.jove.com/video/2490/c-elegans-positive-butanone-learning-short-term-long-term-associative (Kauffman et al. 2010, 2011; Zwoinska et al. 2013).

As in the learning assay used during selection, the postselection learning assays involved conditioning with 2 μl of 10% butanone. However, in addition to postconditioning chemotaxis we also scored naive chemotaxis, to construct a learning index (LI). As described above, the assays differed between Experiments 1 and 2. In Experiment 1, the attraction to butanone in both the naive- and in the postconditioning chemotaxis assays was tested in the presence of benzaldehyde (the same procedure as in the selection protocol). In Experiment 2, however, chemotaxis was instead measured in the presence of solvent (1 μl of 96% ethanol). Importantly, for both experiments new worms were used in every assay, as described in the original olfactory butanone learning protocol (Kauffman et al. 2010, 2011). Once the assays were terminated, worms were killed by reversing plates over a few drops of chloroform and the number of worms within each odorant spot was hand counted. The standard way to measure attraction to odorants is to use the chemotaxis index (CI). For Experiment 1, it was calculated as $CI = (n_{\text{butanone half}} - n_{\text{benzaldehyde half}}) / (n_{\text{butanone half}} + n_{\text{benzaldehyde half}})$, and for Experiment 2 as: $CI = (n_{\text{butanone spot}} - n_{\text{ethanol spot}}) / (n_{\text{total on a plate}} - n_{\text{place of origin}})$, where n refers to the number of worms. Therefore, we always measured CI in reference to butanone. The difference in formulas between the two experiments reflects the different design of the assay plates (see Fig. S2), while in Experiment 1 worms had to choose between two sides of an assay plate in Experiment 2 they had to approach either of two spots on a plate. Note that in both cases CI can range from 1 (perfect attraction) to −1 (perfect aversion) with 0 indicating no preference. For both experiments, the LI was calculated as the difference between CI_{naive} and $CI_{\text{postconditioning}}$.

In each experiment we conducted two assays for every combination of age (days 2 and 7 of adulthood) and sex for each line, giving 2 treatments × 3 replicate lines × 2 sexes × 2 ages × 2 replicate runs = 48 assays. Pairs of upward-selected and one downward-selected lines (blocks) selected on the same day were also tested on the same day. Worms were tested at ages 2 and 7 of adulthood because previous studies suggest that worms senesce at the age of 7 days and therefore we were interested in the senescence of sex-specific learning performance. To avoid the mixing of adults with progeny, the populations of worms to be tested at day 7 of adulthood were transferred daily to new plates by suspending them in M9 buffer (Stiernagle 2006), which separated them from their eggs.

FECUNDITY ASSAY

The reproductive output of males and females was measured at days 2 and 5 of adulthood, during the peak of their reproduction (Zwoinska et al. 2013). In female reproductive assays, 30 virgin females were isolated at L4 (the last larval stage) from each

replicate line and each female was paired with two virgin males coming from ancestral populations (giving total of 90 females per treatment). At days 2 and 5 of adulthood, the females were isolated onto new plates to lay eggs (three hours for day 2 and six hours for day 5 of adulthood, due to lower egg production at this day. For day 5 we report output as the number of eggs produced during six hours/two). During the fecundity assays the females were always paired with two-day-old males to control for the effect of male age. In the male reproductive assay, 20 virgin males from each line were isolated individually with two virgin females from the ancestral populations (giving a total of 60 males per treatment). The sample size was lower for males than for females for logistic reasons because the reproduction assays are more difficult to set up for males. At days 2 and 5 of adulthood, each male was paired with eight virgin ancestral females for three hours, where after the males were removed and the females were allowed to lay eggs for another three hours. *Caenorhabditis remanei* males can mate with multiple females within three hours and our results indicate that the average number of females mated by young males varies between 4 and 5. The offspring produced by all females that mated with the focal male were killed with chloroform and hand counted in their last larval stage (L4).

LIFE SPAN ASSAY

The life span assays were set up using worms in their fourth larval stage (L4) from lines age synchronized by hypochlorite treatment. We measured life span for males and females in either mixed-sex or same-sex groups (i.e., mated or virgin worms). In mixed-sex cohorts, the ancestral populations of *C. remanei* SP8 were used as background "tester" sex and the sex ratio was adjusted to 50:50 throughout the assay based on the mortality of the focal sex. The total number of worms per experimental plate was 10 (either 10 same-sex individuals or five males and five females). The worms were transferred to the new Petri plates (35 mm diameter seeded with 200 μl of *E. coli* OP50 1 pUC4K) every second day. For same-sex combinations we had the initial number of 240 focal individuals (24 plates × 10 focal individuals on each plate) and for mixed-sex combinations 120 focal individuals (24 plates × 5 focal individuals on each plate). The higher number for same-sex combinations was to compensate for higher dispersal of virgin worms (unmated *Caenorhabditis*, in particular males, tend to wander off the food lawn and die on the plate edges). All missing worms were censored. A few plates lost early in the experiment were excluded.

LOCOMOTORY ACTIVITY ASSAY

We measured male and female activity using a modified version of a previously described body bends assay (Koelle 2006). *Caenorhabditis* move by generating sinusoidal waves, that is, bends, therefore the number of bends determine the distance covered (Gray et al. 2005). However, the same distance may reflect different exploratory strategies depending on the number of turns and reversals. We isolated individual mated worms on 35 mm plates seeded with 200 μl of *E. coli* OP50 1 (pUC4K) for 2.5–3 h. After this time, we measured the number of body bends defined as the moment when a pharynx of a worm reached a maximum bend in one direction. We measured both forward and backward bends over a period of 3 min for every individual worm at days 2, 4, 6, 8, and 10 of adulthood. In total we measured bends for 12 males and females per line giving 36 males and females per treatment and age.

STATISTICAL ANALYSIS

All analyses were conducted in *R* version 3.2.0 (R Core Team 2015). Except for the survival analyses, we used mixed-effect models (function lmer) in a maximum-likelihood approach implemented in the package *lme4* (Bates et al. 2015). To perform significance tests on the fixed parameter mixed models were followed by the analysis of deviance table (function *Anova*) with type III Wald chi-square tests and (contrasts set to contr.sum corresponding to deviation coding for categorical variables) using the *car* package (Fox and Weisberg 2011). Consequently, we report fixed effects parameter estimates as coming from Wald chi-square tests and random effects parameter estimates as coming from mixed models.

The data from the learning, fitness, and locomotory assays were standardized by applying *Z* score transformation on the response variables prior to the analyses as well as for graphical presentation (for raw data see Figs. S3–S6). The data from the learning assays were standardized between the sexes and treatments. We fitted adult age, sex, and treatment as fixed effects in the full factorial model, with replicate population as a random effect. Age was centered at day 2 of adulthood. For statistical evaluation of learning performance, we employed two different analytical approaches: first, with LI as a response, and second with postconditioning chemotaxis as a response and naive chemotaxis as a covariate. We did not include the ancestral populations in our learning performance analyses because they were not maintained under the same conditions as the selection lines and also differed in the effective population size.

The data from the reproductive performance assays were standardized by applying *Z* score transformation within the sexes and between the treatments (therefore, males and females cannot be directly compared). Age was centered at day 2 of adulthood. We fitted adult age and treatment as fixed effects in a full factorial model and individual and replicate population as random effects. We analyzed locomotory activity using body bends per minute as the response (after *Z* score transformation), adult age (centered at day 2 of adulthood) and treatment as crossed fixed effects, and

replicate population a random effect (new individuals were tested at each age).

For survival analysis we employed the *coxme* package that allows fitting Cox proportional hazard models with Gaussian random effects (Therneau 2015). As fixed effects we fitted treatment, sex, and mating status, and as random effects we fitted replicate populations.

Results

NAIVE ATTRACTION AND LEARNING PERFORMANCE

Experiment 1

The results from the experiment using the selection protocol (Experiment 1) showed that downward-selected lines exhibited a significantly lower naive attraction to butanone than upward-selected lines when benzaldehyde was present ($\chi^2 = 42.022$, df $= 1$, $P < 0.001$) (Fig. 1A and B). Neither sex nor age had an effect on the levels of naive attraction to butanone (Table 1). In the model with postconditioning chemotaxis as the response variable and naive attraction as a covariate, we found a significant treatment × sex interaction ($\chi^2 = 9.085$, df $= 1$, $P = 0.003$). When sexes were analyzed separately males from upward-selected lines exhibited higher postconditioning attraction to butanone than males from downward-selected lines ($\chi^2 = 26.663$, df $= 1$, $P < 0.001$) (Fig. 1D), and the same was true for females although the difference was less pronounced ($\chi^2 = 10.613$, df $= 1$, $P = 0.001$) (Fig. 1C). Naive attraction was not a significant predictor in any of the models (Table 1). That would suggest that both males and females from upward-selected lines were better learners than males and females from downward-selected lines. When assessing learning performance using the LI (calculated as a difference between postconditioning and naive chemotaxis) we also found a treatment × sex interaction ($\chi^2 = 7.847$, df $= 1$, $P = 0.005$). We investigated males and females separately and found that males from downward-selected lines performed worse than males from upward-selected lines ($\chi^2 = 33.193$, df $= 1$, $P < 0.001$). Strikingly, that was not the case for females, the selected sex, where we found no difference between upward and downward lines in learning performance ($\chi^2 = 2.498$, df $= 1$, $P = 0.114$) (Fig. 1E and F).

Experiment 2

Analyzing naive chemotaxis in Experiment 2, we found no significant effect of treatment on naive attraction to butanone when benzaldehyde was absent ($\chi^2 = 2.601$, df $= 1$, $P = 0.107$). We also identified a marginally nonsignificant interaction between day and sex ($\chi^2 = 3.700$, df $= 1$, $P = 0.054$): as animals aged naive attraction decreased more in females than males (Fig. 2A and B). In the model with postconditioning chemotaxis as the response and naive chemotaxis as a covariate, we found an interaction between age and treatment ($\chi^2 = 17.719$, df $= 1$, $P < 0.001$): postconditioning chemotaxis decreased more with age in upward-selected lines (Fig. 2C and D). Sex but not naive chemotaxis was a significant predictor in this model ($\chi^2 = 11.646$, df $= 1$, $P < 0.001$ and $\chi^2 = 0.503$, df $= 1$, $P = 0.478$) as males had higher postconditioning attraction to butanone than females (Fig. 2C and D). When assessing learning performance using the LI, we found an effect of the selection treatment, as downward-selected lines performed worse ($\chi^2 = 19.527$, df $= 1$, $P < 0.001$) and also an effect of sex, as males performed better than females ($\chi^2 = 10.185$, df $= 1$, $P = 0.001$) but no interaction (Fig. 2E and F). Overall both approaches suggested better learning performance of upward-selected lines and the lack of significant treatment × sex interactions (Table 1).

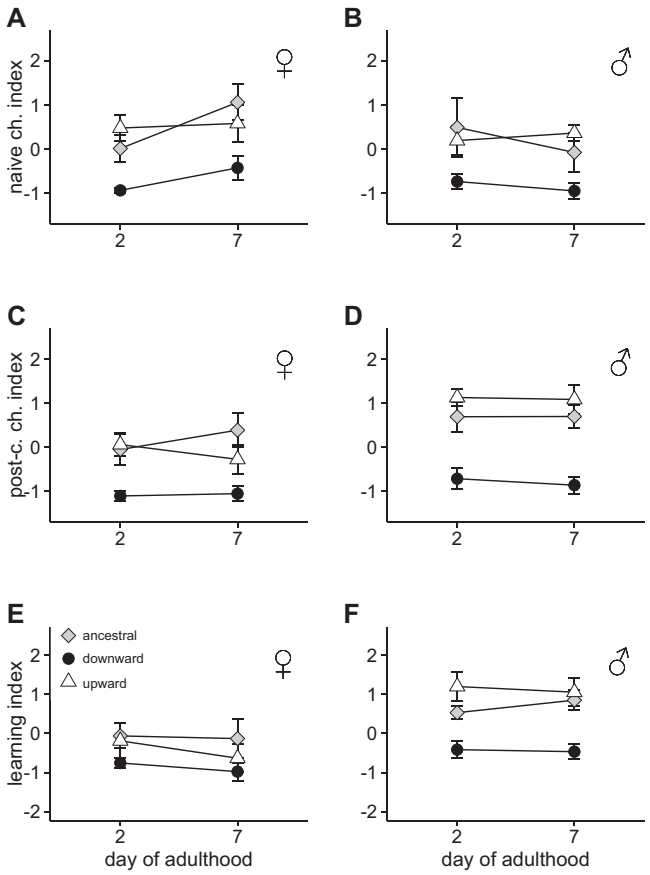

Figure 1. Performance in the selection protocol (Experiment 1). (A) and (B) Naive chemotaxis index; (C) and (D) postconditioning chemotaxis index; (E) and (F) learning index calculated as a difference between post- and naive chemotaxis indices. Left column females, right column males. Data (means ± SEM) were standardized by applying *Z* score transformation between the sexes (and treatments) meaning that the magnitude of response can be directly compared between the sexes. For illustrative purpose we included the ancestral population. See Figure S3 for raw data.

Table 1. For learning performance—Experiments 1 and 2, locomotory activity, and reproductive performance assays, we report mixed-effect models (for random effects) and type III Wald chi-square tests (for fixed effects) (see Materials and methods).

Experiment 1: selection learning protocol			
Analysis of deviance table (type III Wald chi-square tests)			
Response: Naive chemotaxis			
	Chi-square	df	*P*
Intercept	7.6795	1	0.006
Day	0.703	1	0.402
Sex	1.466	1	0.226
Treatment	42.022	1	<0.001
Random effects			
	Variance		Std. dev.
Treatment: replicate	0.007		0.081
Residual	0.482		0.694
Response: postconditioning chemotaxis			
	Chi-square	df	*P*
Intercept	0.2788	1	0.598
Day	0.630	1	0.427
Sex	23.768	1	<0.001
Treatment	36.796	1	<0.001
Naive chemotaxis	0.046	1	0.829
Treatment: sex	9.085	1	0.003
Random effects			
	Variance		Std. dev.
Treatment: replicate	0.010		0.100
Residual	0.275		0.525
Response: postconditioning chemotaxis (males)			
	Chi-square	df	*P*
Intercept	6.847	1	0.009
Day	0.188	1	0.665
Treatment	26.663	1	<0.001
Naive chemotaxis	0.225	1	0.635
Random effects			
	Variance		Std. dev.
Treatment: replicate	0.023		0.153
Residual	0.270		0.520
Response: postconditioning chemotaxis (females)			
	Chi-square	df	*P*
Intercept	3.974	1	0.046
Day	0.430	1	0.512
Treatment	10.613	1	0.001
Naive chemotaxis	0.005	1	0.942
Random effects			
	Variance		Std. dev.
Treatment: replicate	0.000	0.000	
Residual	0.273	0.525	
Response: learning index			
	Chi-square	df	*P*
Intercept	7.556	1	0.006
Day	1.176	1	0.278
Sex	2.292	1	0.130
Treatment	2.617	1	0.106
Treatment: sex	7.847	1	0.005

(*Continued*)

Table 1. Continued.

Random effects				
	Variance		Std. dev.	
Treatment: replicate	0.000	0.000		
Residual	0.438	0.662		
Response: learning index (males)				
	Chi-square	df		*P*
Intercept	7.879	1		0.005
Day	0.130	1		0.718
Treatment	33.193	1		<0.001
Random effects				
	Variance		Std. dev.	
Treatment: replicate	0.000		0.000	
Residual	0.411		0.641	
Response: learning index (females)				
	Chi-square	df		*P*
Intercept	2.549	1		0.110
Day	1.338	1		0.247
Treatment	2.498	1		0.114
Random effects				
	Variance		Std. dev.	
Treatment: replicate	0.000		0.000	
Residual	0.459		0.677	
Experiment 2: butanone olfactory learning protocol				
Analysis of deviance table (type III Wald chi-square tests)				
Response: naive chemotaxis				
	Chi-square	df		*P*
Intercept	16.054	1		<0.001
Day	32.108	1		<0.001
Sex	5.853	1		0.016
Treatment	2.601	1		0.107
Day: sex	3.700	1		0.054
Random effects				
	Variance		Std. dev.	
Treatment: replicate	0.000		0.000	
Residual	0.530		0.728	
Response: postconditioning chemotaxis				
	Chi-square	df		*P*
Intercept	21.844	1		<0.001
Day	35.196	1		<0.001
Sex	11.646	1		<0.001
Treatment	87.655	1		<0.001
Naive chemotaxis	0.503	1		0.478
Treatment: day	17.719	1		<0.001
Random effects				
	Variance		Std. dev.	
Treatment: replicate	0.000		0.000	
Residual	0.198		0.445	
Response: learning index				
	Chi-square	df		*P*
Intercept	0.040	1		0.841
Day	0.080	1		0.777
Sex	10.185	1		0.001
Treatment	19.527	1		<0.001

(*Continued*)

Table 1. Continued.

Random effects				
	Variance		Std. dev.	
Treatment: replicate	0.000		0.000	
Residual	0.604		0.773	
Reproductive performance				
Analysis of deviance table (type III Wald chi-square tests)				
Response: number of larvae (females)				
	Chi-square	df		*P*
Intercept	22.100	1		<0.001
Day	226.974	1		<0.001
Treatment	0.004	1		0.948
Random effects				
	Variance		Std. dev.	
Treatment: replicate: ID	0.118		0.343	
Treatment: replicate	0.065		0.255	
Residual	0.500		0.707	
Response: number of larvae (males)				
	Chi-square	df		*P*
Intercept	0.129	1		0.720
Day	0.212	1		0.646
Treatment	25.656	1		<0.001
Random effects				
	Variance		Std. dev.	
Treatment: replicate: ID	0.000		0.000	
Treatment: replicate	0.000		0.000	
Residual	0.838		0.915	
Survival				
Cox proportional hazards models with Gaussian random effects				
Response: time until death (both sexes, reproducing and virgins)				
	Exp(coef)	*Z*		*P*
Sex	0.309	−7.56		<0.001
Treatment	1.124	0.42		0.67
Mating status	0.108	−16.38		<0.001
Sex: treatment	1.010	0.05		0.96
Sex: mating	2.310	4.09		<0.001
Treatment: mating	0.676	−2.19		0.028
Treat: sex: mating	2.044	2.51		0.012
Random effects				
	Variance		Std. dev.	
Treatment: replicate	0.087		0.295	
Response: survival (virgins)				
	Exp(coef)	*Z*		*P*
Sex	0.684	−2.79		0.005
Treatment	0.732	−0.70		0.480
Sex: treatment	2.345	4.32		<0.001
Random effects				
	Variance		Std. dev.	
Treatment: replicate	0.275		0.524	
Response: survival (reproducing)				
	Exp(coef)	*Z*		*P*
Sex	0.374	−8.71		<0.001
Treatment	1.012	0.07		0.94

(*Continued*)

Table 1. Continued.

Random effects				
	Variance		Std. dev.	
Treatment: replicate	0.030		0.174	
Response: survival (downward-selected, virgins)				
	Exp(coef)	*Z*		*P*
Sex	0.691	−2.69		0.007
Random effects				
	Variance		Std. dev.	
Treatment: replicate	0.630		0.794	
Response: survival (downward-selected, reproducing)				
	Exp(coef)	*Z*		*P*
Sex	0.424	−5.45		<0.001
Random effects				
	Variance		Std. dev.	
Treatment: replicate	0.000		0.009	
Response: survival (upward-selected, virgins)				
	Exp(coef)	*Z*		*P*
Sex	1.610	3.34		<0.001
Random effects				
	Variance		Std. dev.	
Treatment: replicate	0.078		0.280	
Response: survival (upward-selected, reproducing)				
	Exp(coef)	*Z*		*P*
Sex	0.310	−7.13		<0.001
Random effects				
	Variance		Std. dev.	
Treatment: replicate	0.115		0.339	
Locomotory activity				
Analysis of deviance table (type III Wald chi-square tests)				
Response: body bends per minute (males)				
	Chi-square	df		*P*
Intercept	358.066	1		<0.001
Day	3.023	1		0.082
Day^2	20.084	1		<0.001
Day^3	17.302	1		<0.001
Treatment	4.214	1		0.040
Random effects				
	Variance		St. dev.	
Treatment: replicate	0.009		0.093	
Residual	0.324		0.570	
Response: body bends per minute (females)				
	Chi-square	df		*P*
Intercept	3.8417	1		0.050
Day	230.726	1		<0.001
Treatment	6.547	1		0.011
Random effects				
	Variance		Std. dev.	
Treatment: replicate	0.000		0.000	
Residual	0.221		0.470	

For survival analysis we report Cox proportional hazards models with Gaussian random effects.

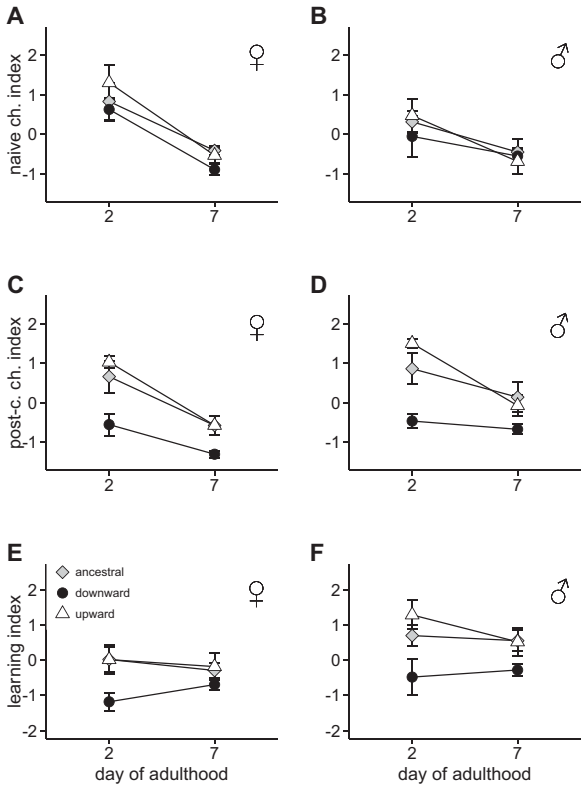

Figure 2. Performance in the original protocol (Experiment 2). (A) and (B) Naive chemotaxis index; (C) and (D) postconditioning chemotaxis index; (E) and (F) learning index calculated as a difference between post- and naive chemotaxis indices. Left column females, right column males. Data (means ± SEM) were standardized by applying *Z* score transformation between the sexes (and treatments) meaning that the magnitude of response can be directly compared between the sexes. For illustrative purpose we included the ancestral population. See Figure S4 for raw data.

FECUNDITY

Upward- and downward-selected females did not differ in reproductive output ($\chi^2 = 0.004$, df $= 1$, $P = 0.948$) (Fig. 3A). In contrast, males from downward-selected lines sired significantly fewer progeny than males from upward-selected lines ($\chi^2 = 25.655$, df $= 1$, $P < 0.001$) (Fig. 3B). Females, but not males, showed signs of reproductive ageing by day 5 of adulthood ($\chi^2 = 226.974$, df $= 1$, $P < 0.001$ and $\chi^2 = 0.212$, df $= 1$, $P = 0.646$).

SURVIVAL

In the full model with found a three-way interaction between sex, treatment, and mating status ($z = 2.51$, $P = 0.012$). This was followed up with analysis on virgin and reproducing worms separately. For virgin worms we found a significant sex × treatment interaction ($z = 4.32$, $P < 0.001$): although the risk of death was higher for downward-selected virgin females than for upward-selected virgin females (median life span 28 days vs. 30 days) (Fig. 4D), the opposite was true for males (median life span 28 vs. 26 days) (Fig. 4B). For reproducing worms only the main effect of sex was significant ($z = -8.71$, $P < 0.001$) because females had a higher risk of death than males (median life span 14 vs. 18 days) (Fig. 4A and C). Moreover, in downward-selected lines, males had a lower risk of the death than females for both virgin and reproducing worms ($z = -2.69$, $P = 0.007$ and $z = -5.45$, $P < 0.001$) (median life span for males: 28 for virgin and 18 reproducing worms, for females: 28 and 14). However, in upward-selected lines, males had a lower risk of death than females only in reproducing worms ($z = -7.13$, $P < 0.001$) while the opposite was true for virgins ($z = 3.34$, $P < 0.001$) (median life span for males: 26 for virgin and 18 for reproducing worms, for females: 30 and 14).

LOCOMOTORY ACTIVITY

Male locomotory activity differed between the treatments as males from upward-selected female lines showed lower activity levels than males from downward-selected female lines ($\chi^2 = 4.214$, df $= 1$, $P = 0.040$) (Fig. 5B). The number of body bends decreased significantly from day 2 to day 10 of adulthood in both treatments (day: $\chi^2 = 3.023$, df $= 1$, $P = 0.082$; day^2: $\chi^2 = 20.084$, df $= 1$, $P < 0.001$; day^3: $\chi^2 = 17.302$, df $= 1$, $P < 0.001$). Similarly, upward-selected females showed generally lower activity levels than downward-selected females ($\chi^2 = 6.547$, df $= 1$, $P = 0.011$) (Fig. 5A), and the number of body bends decreased with age ($\chi^2 = 230.726$, df $= 1$, $P < 0.001$).

Discussion

Sex-specific selection on cognitive performance can result in divergent correlated evolution of life-history and behavioral traits in males and females and, therefore, can shape the evolution of sex-specific life histories. However, such evolution can be constrained by intersexual genetic correlations. Here, we investigated the sex-specific fitness consequences of learning evolution by selecting on improved and decreased olfactory learning performance in females of the nematode *C. remanei*, and measured learning, reproductive performance, life span, and locomotory activity in both sexes. The correlated response to selection on females was strong and significant in males in the two different experiments regardless of the analytical approach (LI or postconditioning chemotaxis corrected for naive response). However, the direct female response to selection was generally weaker suggesting sex-specific genetic architecture for learning performance. Males also performed better than females in both learning assays at two different ages. One of our key findings is that the males from downward-selected female lines were more active and had longer intrinsic life span (i.e., lived longer as virgins in the absence of the costs of reproduction) but sired fewer progeny than males from upward-selected

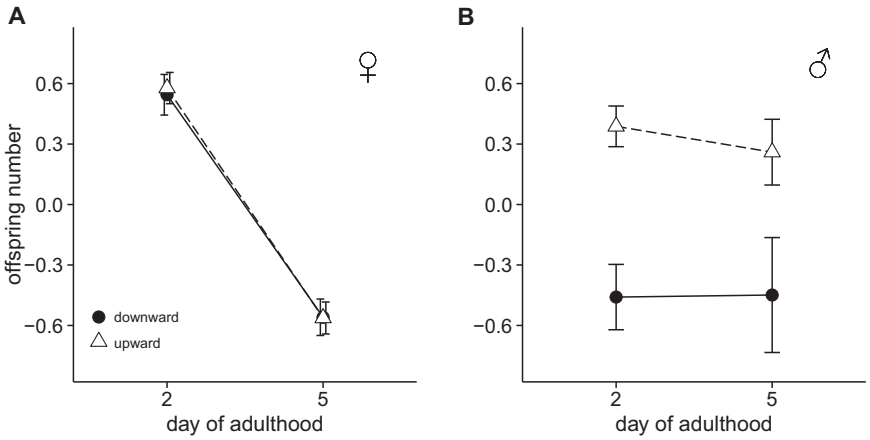

Figure 3. Male (B) and female (A) reproductive performance measured as the number of L4 larvae produced. Data (means ± SEM) were standardized by applying *Z* score transformation within the sexes and between treatments meaning that the magnitude of response can only be compared between the treatments within a given sex. See Figure S5 for raw data.

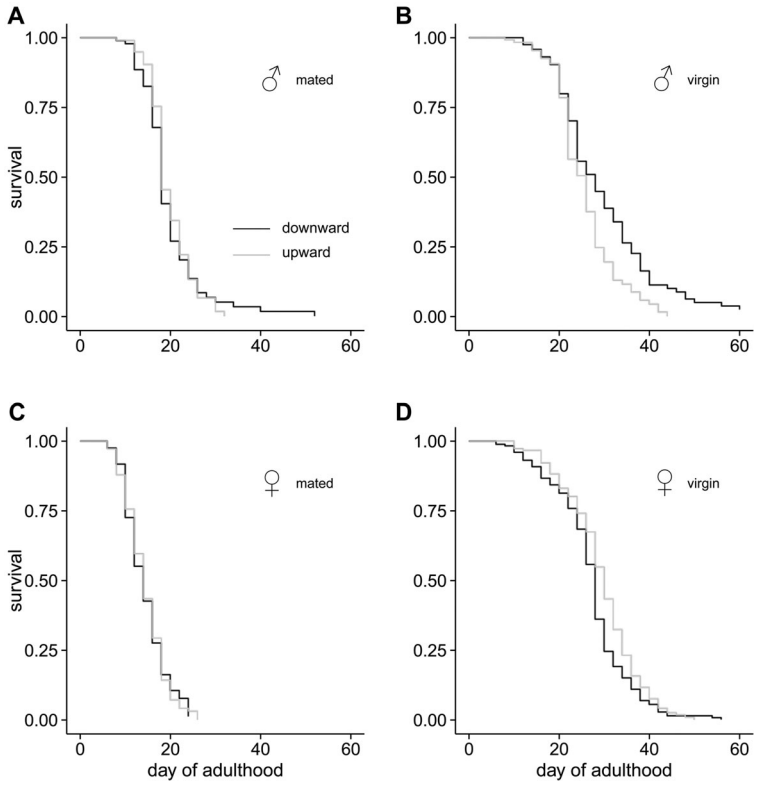

Figure 4. Kaplan–Meier survival curves of reproducing males (A), virgin males (B), reproducing females (C), and virgin females (D) from upward- (grey lines) and downward-selected (black lines) lines.

female lines. In stark contrast, the reproductive performance of females was unaffected by selection for learning performance, although the downward-selected females had shorter intrinsic life span and higher activity levels than upward-selected females. Although the virgin upward-selected females outlived virgin males from upward-selected female lines, the opposite was true for the downward-selected worms. We found no life span difference between reproducing worms.

Based on the mating biology of our species (Barr and Garcia 2006) and findings linking male reproductive and learning performance (Hollis and Kawecki 2014), we predicted that learning performance would be more critical for male than for female fitness. Indeed, we found a fitness cost of decreased learning performance in the males from downward-selected female lines, and a lack thereof in females. Currently we are unable to attribute reduced reproductive performance to either lower fecundity or

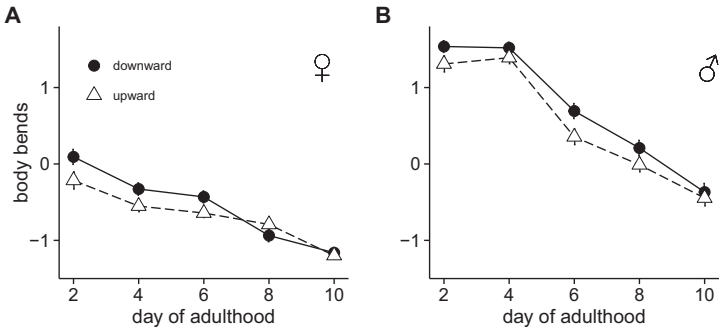

Figure 5. Locomotory activity or females (A) and males (B) from upward- (dashed line, triangles) and downward-selected (solid line, circles) lines. Data (means ± SEM) were standardized by applying *Z* score transformation between the sexes (and treatments) meaning that the magnitude of response can be directly compared between the sexes. See Figure S6 for raw data.

impaired mate search, which requires further research. Importantly, the males from downward-selected female lines showed higher locomotory activity levels and longer intrinsic life span than the males from upward-selected female lines, suggesting that the males from downward-selected female lines were in good physical health. In our previous study, we found that young females outperformed males in an olfactory learning task (Zwoinska et al. 2013) but here both young and old females from both treatments scored worse than males. We used a different type of nematode growth medium in the previous study, which could have influenced the life history of our experimental animals.

Selection on learning performance in females also resulted in sex-specific evolution of virgin life span as a correlated response. Previous studies on *Drosophila* that documented life span costs of improved learning and long-term memory measured virgin life span (Burger et al. 2008; Lagasse et al. 2012), and therefore shortened virgin life span of males from upward-selected female lines as compared to males from downward-selected female lines corroborates these findings. However, the upward-selected females had longer intrinsic life span than the downward-selected females, revealing sex-specific genetic architecture for life span and cognition in *C. remanei*. This is in line with recent studies that showed high levels of sex-specific genetic variation for life span in *D. melanogaster* (Lehtovaara et al. 2013) and in *C. remanei* (Chen and Maklakov 2014). The fact that life span of reproducing upward- and downward-selected worms was indistinguishable shows that costs of reproduction between the experimental lines can mask trade-offs between intrinsic life span and cognitive traits. Therefore, we suggest that life span should be measured on both virgin and reproducing individuals when we are interested in the genetic trade-off between life span and other life-history and/or behavioral traits.

In *Caenorhabditis* nematodes males usually outlive females/hermaphrodites (McCulloch and Gems 2003), which likely results from the substantial costs of intersexual encounters paid by females/hermaphrodites and to a lesser extent by males (Gems and Riddle 1996; Maures et al. 2014; Shi and Murphy 2014; Zwoinska et al. 2014). In the ancestral population, virgin females live longer than virgin males (Lind et al. 2015) and this corresponds to our findings in populations selected for improved learning performance. However, in the downward-selected lines, the sexual dimorphism in virgin life span was reversed, as virgin males outlived virgin females. To the best of our knowledge, this is the first study showing how selection on learning performance, or any other trait for that matter, results in the reversal of sex difference in life span. Our results show that sex differences in intrinsic life span can evolve rapidly in the absence of differences in sex-specific mortality, solely as a result of sex-specific genetic trade-offs between life span and a cognitive trait. Classic theory maintains that the sex that experiences the highest rate of extrinsic mortality should evolve shorter intrinsic life span (Williams 1957); however, our finding supports the idea that sex differences in life span can evolve because of adaptive sex-specific selection (Maklakov and Lummaa 2013; Chen and Maklakov 2014). In terms of mechanisms governing the reversal in sexual dimorphism in life span we would consider two possibilities; first, sex-specific genetic costs of learning performance, and second, behavioral changes following selection that affect life span in a sex-specific manner. Studies on insects have documented evolutionary costs of learning ability in terms of reduced life span (Burger et al. 2008; Lagasse et al. 2012) and similar costs may have played a role in our lines resulting in increased life span of males from downward-selected female lines. However, the opposing response in females suggests positive genetic correlation between life span and learning performance in this sex.

These results are broadly in line with recent work on intralocus conflict over optimal life span in other taxa (Bilde et al. 2009; Lewis et al. 2011; Berg and Maklakov 2012; Berger et al. 2014). For example, in the seed beetle *Callosobruchus maculatus* selection on reduced life span decreases female fitness but increases male fitness (Berg and Maklakov 2012; Berger et al. 2014). In our study, males from downward-selected female lines had lower

reproductive performance and longer life span but downward-selected females had shorter life span while their reproductive performance was unaffected. However, reduction in learning performance in downward-selected females is likely to reduce their fitness in the environment where they would have to locate food or avoid getting in contact with pathogens. Therefore, our findings are broadly in agreement with the idea that males and females have different life-history syndromes and that males are more likely to sacrifice longevity for reproductive success (Bonduriansky et al. 2008; Adler and Bonduriansky 2014) resulting in the negative genetic correlation between life span and reproductive performance in males but not in females.

As our lines also differed in naive attraction to odorants in one of the experiments, we have to consider the contribution of sensory perception to the observed patterns. Importantly, Experiment 2, where we found no significant differences in naive attraction to odorants demonstrates that the differences in naive attraction alone do not explain differences in learning performance between our lines. In fact, clear separation between the different mechanisms underlying performance in cognitive tasks proves difficult, which recently stirred up a discussion on the interpretation of studies measuring learning and problem solving (Healy and Rowe 2013; Barrett 2014; Kolm 2014; Quinn et al. 2014; Rowe and Healy 2014; Thornton 2014; Thornton et al. 2014). Some argue that identifying cognitive traits behind broadly defined cognitive performance and separating them from noncognitive traits is crucial for the development of the field (Healy and Rowe 2013; Rowe and Healy 2014; Thornton 2014; Thornton et al. 2014). Others maintain that cognitive ability can be measured and studied to advance our understating of the evolutionary forces underlying cognitive function even if traits that contribute to cognitive ability can be used in other contexts (Barrett 2014; Kolm 2014; Quinn et al. 2014). We would like to add that, ultimately, such decisions depend on the question. Here we asked whether selection on cognitive ability, in a form of learning performance, results in the evolution of sex-specific life histories because of sex-specific trade-offs and between-sex between-trait genetic correlations. Whether the observed evolutionary change in learning performance is driven by a purely cognitive trait, or by a trait that is also used in different contexts, such as perception, was not the focus of this study. Therefore, such interpretational problems that are inherent to a relatively new and multidisciplinary field do not change the main conclusion of our study on the sex-specific link between learning performance and life-history traits.

Conclusions

Selection on cognitive ability is often postulated to play an important role in phenotypic evolution (Dukas and Ratcliffe 2009; Shettleworth 2010). However, the theoretical possibility that selection on cognitive ability can result in the evolution of sex-specific life histories merely via sex-specific genetic correlations with life-history traits remains largely unexplored (Burger et al. 2008; Hollis and Kawecki 2014). Recent studies in different taxa suggest that sexually antagonistic selection shapes the evolution of male and female life span (Lewis et al. 2011; Berg and Maklakov 2012). Because life span is genetically correlated with behavioral, morphological, and life-history traits, sexually antagonistic selection on life span can result in multivariate intralocus conflict over suites of functionally integrated traits, so-called life-history syndromes (Berger et al. 2014). The corollary is that selection on one complex trait characterized by genetic correlations with life span, such as cognitive ability (Burger et al. 2008; Lagasse et al. 2012), should lead to the evolution of sex-specific life histories. Here we provided direct experimental evidence for this proposition by showing that selection on learning performance resulted in the evolution of sex-specific life histories. Poor learning performance was associated with increased intrinsic life span in males but decreased intrinsic life span in females resulting in the reversal of sexual dimorphism in this trait. Our results imply that future studies should focus on the physiological and behavioral mechanisms that underlie sex differences in the cost of learning performance. More broadly, our findings suggest that cognitive ability is genetically integrated in sex-specific life-history syndromes and should be routinely measured alongside life history and morphology.

DATA ARCHIVING

The doi for our data is 10.5061/dryad.r4g6p.

LITERATURE CITED

Abbott, J. K., S. Bedhomme, and A. K. Chippindale. 2010. Sexual conflict in wing size and shape in *Drosophila melanogaster*. J. Evol. Biol. 23:1989–1997.

Adler, M. I., and R. Bonduriansky. 2014. Why do the well-fed appear to die young? A new evolutionary hypothesis for the effect of dietary restriction on lifespan. Bioessays 36:439–450.

Bachevalier, J., and C. Hagger. 1991. Sex differences in the development of learning abilities in primates. Psychoneuroendocrinology 16:177–188.

Barr, M. M., and L. R. Garcia. 2006. Male mating behavior in WormBook, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.78.1, http://www.wormbook.org

Barrett, L. 2014. What counts as (non) cognitive? A comment on Rowe and Healy. Behav. Ecol. 25:1293–1294.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity (Edinb.) 2:349–368.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Ime4:Linear mixed-effects models using Eigen and S4. R package version 1.1–9. Available athttps://CRAN.R-project.org/package=lme4.

Bedhomme, S., A. K. Chippindale, N. G. Prasad, M. Delcourt, J. K. Abbott, M. A. Mallet, and H. D. Rundle. 2011. Male-limited evolution suggests no extant intralocus sexual conflict over the sexually dimorphic cuticular hydrocarbons of *Drosophila melanogaster*. J. Genet. 90:443–452.

Berg, E. C., and A. A. Maklakov. 2012. Sexes suffer from suboptimal lifespan because of genetic conflict in a seed beetle. Proc. Biol. Sci. 279:4296–4302.

Berger, D., E. C. Berg, W. Widegren, G. Arnqvist, and A. A. Maklakov. 2014. Multivariate intralocus sexual conflict in seed beetles. Evolution 68:3457–3469.

Bilde, T., A. A. Maklakov, K. Meisner, L. la Guardia, and U. Friberg. 2009. Sex differences in the genetic architecture of lifespan in a seed beetle: extreme inbreeding extends male lifespan. BMC Evol. Biol. 9:33.

Bonduriansky, R., and S. F. Chenoweth. 2009. Intralocus sexual conflict. Trends Ecol. Evol. 24:280–288.

Bonduriansky, R., A. Maklakov, F. Zajitschek, and R. Brooks. 2008. Sexual selection, sexual conflict and the evolution of ageing and life span. Funct. Ecol. 22:443–453.

Burger, J. M., M. Kolss, J. Pont, and T. J. Kawecki. 2008. Learning ability and longevity: a symmetrical evolutionary trade-off in *Drosophila*. Evolution 62:1294–1304.

Carazo, P., D. W. Noble, D. Chandrasoma, and M. J. Whiting. 2014. Sex and boldness explain individual differences in spatial learning in a lizard. Proc. Biol. Sci. 281:20133275.

Chen, H. Y., and A. A. Maklakov. 2012. Longer life span evolves under high rates of condition-dependent mortality. Curr. Biol. 22:2140–2143.

Chanov, J. R., W. K. So,K. L. Chan, and K. L. Chow. The species, sex, and stage specificity of a *Caenorhabditis sex* pheromone. Natl. Acad. Sci. USA 104:6730–6735.

Chasnov, J. R., W. K. So, K. L. Chan, and K. L. Chow. 2007. The species, sex, and stage specificity of a *Caenorhabditis* sex pheromone. Proc. Natl. Acad. Sci. USA 104:6730–6735.

———. 2014. Condition dependence of male mortality drives the evolution of sex differences in longevity. Curr. Biol. 24:2423–2427.

Chippindale, A. K., J. R. Gibson, and W. R. Rice. 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. Proc. Natl. Acad. Sci. USA 98:1671–1675.

Colbert, H. A., and C. I. Bargmann. 1995. Odorant-specific adaptation pathways generate olfactory plasticity in *C. elegans*. Neuron 14:803–812.

Cole, E. F., and J. L. Quinn. 2012. Personality and problem-solving performance explain competitive ability in the wild. Proc. Biol. Sci. 279:1168–1175.

Cole, E. F., J. Morand-Ferron, A. E. Hinks, and J. L. Quinn. 2012. Cognitive ability influences reproductive life history variation in the wild. Curr. Biol. 22:1808–1812.

Dalla, C., and T. J. Shors. 2009. Sex differences in learning processes of classical and operant conditioning. Physiol. Behav. 97:229–238.

Delcourt, M., M. W. Blows, J. D. Aguirre, and H. D. Rundle. 2012. Evolutionary optimum for male sexual traits characterized using the multivariate Robertson-Price Identity. Proc. Natl. Acad. Sci. USA 109:10414–10419.

Diaz, S. A., J. Lindstrom, and D. T. Haydon. 2008. Basic demography of *Caenorhabditis remanei* cultured under standard laboratory conditions. J. Nematol. 40:167–178.

Dukas, R., and E. A. Bernays. 2000. Learning improves growth rate in grasshoppers. Proc. Natl. Acad. Sci. USA 97:2637–2640.

Dukas, R., and J. J. Duan. 2000. Potential fitness consequences of associative learning in a parasitoid wasp. Behav. Ecol. 11:536–543.

Dukas, R., and J. M. Ratcliffe. 2009. Cognitive ecology II. The University of Chicago Press, Chicago.

Fox, J., and S. Weisberg. 2011. An R companion to applied regression. 2nd ed. Sage, Thousand Oaks, CA.

Fritzsche, K., N. Timmermeyer, M. Wolter, and N. K. Michiels. 2014. Female, but not male, nematodes evolve under experimental sexual coevolution. Proc. Biol. Sci. 281:20140942.

Gems, D., and D. L. Riddle. 1996. Longevity in *Caenorhabditis elegans* reduced by mating but not gamete production. Nature 379:723–725.

Gosden, T. P., and S. F. Chenoweth. 2014. The evolutionary stability of cross-sex, cross-trait genetic covariances. Evolution 68:1687–1697.

Gosden, T. P., K. Shastri, P. Innocenti, and S. F. Chenoweth. 2012. The B matric harbors significant and sex specific constraints on the evolution of multicharacter sexual dimorphism. Evolution 66:2106–2116.

Gray, J. M., J. J. Hill, and C. I. Bargmann. 2005. A circuit for navigation in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA 102:3184–3191.

Guigueno, M. F., D. A. Snow, S. A. MacDougall-Shackleton, and D. F. Sherry. 2014. Female cowbirds have more accurate spatial memory than males. Biol. Lett. 10:20140026.

Harano, T., K. Okada, S. Nakayama, T. Miyatake, and D. J. Hosken. 2010. Intralocus sexual conflict unresolved by sex-limited trait expression. Curr. Biol. 20:2036–2039.

Healy, S. D., and C. Rowe. 2013. Costs and benefits of evolving a larger brain: doubts over the evidence that large brains lead to better cognition. Anim. Behav. 86:E1–E3.

Hollis, B., and T. J. Kawecki. 2014. Male cognitive performance declines in the absence of sexual selection. Proc. Biol. Sci. 281:20132873.

Jonasson, Z. 2005. Meta-analysis of sex differences in rodent models of learning and memory: a review of behavioral and biological data. Neurosci. Biobehav. Rev. 28:811–825.

Jones, C. M., V. A. Braithwaite, and S. D. Healy. 2003. The evolution of sex differences in spatial ability. Behav. Neurosci. 117:403–411.

Jozet-Alves, C., J. Moderan, and L. Dickel. 2008. Sex differences in spatial cognition in an invertebrate: the cuttlefish. Proc. Biol. Sci. 275:2049–2054.

Kauffman, A., L. Parsons, G. Stein, A. Wills, R. Kaletsky, and C. Murphy. 2011. *C. elegans* positive butanone learning, short-term, and long-term associative memory assays. J. Vis. Exp. 49:e2490. doi:10.3791/2490.

Kauffman, A. L., J. M. Ashraf, M. R. Corces-Zimmerman, J. N. Landis, and C. T. Murphy. 2010. Insulin signaling and dietary restriction differentially influence the decline of learning and memory with age. PLoS Biol. 8:e1000372.

Koelle, M. 2006. WormBook *in* A. C. Hart, ed. WormBook.Behavior, WormBook, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.87.1, http://www.wormbook.org.

Kokko, H., and M. D. Jennions. 2008. Parental investment, sexual selection and sex ratios. J. Evol. Biol. 21:919–948.

Kolm, N. 2014. Measuring variation in cognition can be done, but it requires hard empirical work: a comment on Rowe and Healy. Behav. Ecol. 25:1296–1297.

Kotrschal, A., S. D. Buechel, S. M. Zala, A. Corral-Lopez, D. J. Penn, and N. Kolm. 2015. Brain size affects female but not male survival under predation threat. Ecol. Lett. 18:646–652.

Kwan, L., S. Bedhomme, N. G. Prasad, and A. K. Chippindale. 2008. Sexual conflict and environmental change: trade-offs within and between the sexes during the evolution of desiccation resistance. J. Genet. 87:383–394.

Lagasse, F., C. Moreno, T. Preat, and F. Mery. 2012. Functional and evolutionary trade-offs co-occur between two consolidated memory phases in *Drosophila melanogaster*. Proc. Biol. Sci. 279:4015–4023.

Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:292–305.

———. 1981. Models of speciation by sexual selection on polygenic traits. Proc. Natl. Acad. Sci. USA 78:3721–3725.

Lehtovaara, A., H. Schielzeth, I. Flis, and U. Friberg. 2013. Heritability of life span is largely sex limited in *Drosophila*. Am. Nat. 182:653–665.

Lewis, Z., N. Wedell, and J. Hunt. 2011. Evidence for strong intralocus sexual conflict in the Indian meal moth, *Plodia interpunctella*. Evolution 65:2085–2097.

Lind, M. I., M. K. Zwoinska, S. Meurling, H. Carlsson, and A. A. Maklakov. 2015. Sex-specific tradeoffs with growth and fitness following life-span extension by rapamycin in an outcrossing nematode, *Caenorhabditis remanei*. J. Gerontol. A Biol. Sci. Med. Sci. glv174.

Lionaki, E., and N. Tavernakis. 2013. High-throughput and longitudinal analysis of aging and senescent decline in *Caenorhabditis elegans*. Methods Mol Biol. 2013;965:485–500. doi:10.-1007/-978-1-62703-239-1_32.

Lonsdorf, E. V., L. E. Eberly, and A. E. Pusey. 2004. Sex differences in learning in chimpanzees. Nature 428:715–716.

Maklakov, A. A., and V. Lummaa. 2013. Evolution of sex differences in lifespan and aging: causes and constraints. Bioessays 35:717–724.

Mank, J. E., L. Hultin-Rosenberg, M. Zwahlen, and H. Ellegren. 2008. Pleiotropic constraint hampers the resolution of sexual antagonism in vertebrate gene expression. Am. Nat. 171:35–43.

Maures, T. J., L. N. Booth, B. A. Benayoun, Y. Izrayelit, F. C. Schroeder, and A. Brunet. 2014. Males shorten the life span of *C. elegans* hermaphrodites via secreted compounds. Science 343:541–544.

McCulloch, D., and D. Gems. 2003. Evolution of male longevity bias in nematodes. Aging Cell 2:165–173.

Mery, F., and T. J. Kawecki. 2003. A fitness cost of learning ability in *Drosophila melanogaster*. Proc. Biol. Sci. 270:2465–2469.

———. 2004. An operating cost of learning in *Drosophila melanogaster*. Anim. Behav. 68:589–598.

———. 2005. A cost of long-term memory in *Drosophila*. Science 308:1148.

Miller, D. I., and D. F. Halpern. 2014. The new science of cognitive sex differences. Trends Cogn. Sci. 18:37–45.

Nuttley, W. M., K. P. Atkinson-Leadbeater, and D. Van Der Kooy. 2002. Serotonin mediates food-odor associative learning in the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA 99:12449–12454.

Ostojic, I., W. Boll, M. J. Waterson, T. Chan, R. Chandra, S. D. Pletcher, and J. Alcedo. 2014. Positive and negative gustatory inputs affect *Drosophila* lifespan partly in parallel to dFOXO signaling. Proc. Natl. Acad. Sci. USA 111:8143–8148.

Placais, P. Y., and T. Preat. 2013. To favor survival under food shortage, the brain disables costly memory. Science 339:440–442.

Prasad, N. G., S. Bedhomme, T. Day, and A. K. Chippindale. 2007. An evolutionary cost of separate genders revealed by male-limited evolution. Am. Nat. 169:29–37.

Quinn, J. L., E. F. Cole, and J. Morand-Ferron. 2014. Studying microevolutionary processes in cognitive traits: a comment on Rowe and Healy. Behav. Ecol. 25:1297–1298.

R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rice, W. R. 1984. Sex chromosome and the evolution of sexual dimorphism. Evol. Hum. Behav. 38:735–742.

Rowe, C., and S. D. Healy. 2014. Measuring variation in cognition. Behav. Ecol. 25:1287–1292.

Scharer, L., L. Rowe, and G. Arnqvist. 2012. Anisogamy, chance and the evolution of sex roles. Trends Ecol. Evol. 27:260–264.

Shettleworth, S. J. 2001. Animal cognition and animal behaviour. Anim. Behav. 61:277–286.

———. 2010. Cognition, evolution, and behavior. Oxford Univ. Press, New York.

Shi, C., and C. T. Murphy. 2014. Mating induces shrinking and death in *Caenorhabditis* mothers. Science 343:536–540.

Snell-Rood, E. C., G. Davidowitz, and D. R. Papaj. 2011. Reproductive tradeoffs of learning in a butterfly. Behav. Ecol. 22:291–302.

Stiernagle, T. 2006. Maintenance of *C. elegans*, WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.101.1, http://www.wormbook.org.

Therneau, T. M. 2015. coxme: Mixed Effects Cox Models. R package version 2.2-5. http://CRAN.R-project.org/package=coxme

Thornton, A. 2014. How and why are some species so smart? A comment on Rowe and Healy. Behav. Ecol. 25:1294–1295.

Thornton, A., J. Isden, and J. R. Madden. 2014. Toward wild psychometrics: linking individual cognitive differences to fitness. Behav. Ecol. 25:1299–1301.

Torayama, I., T. Ishihara, and I. Katsura. 2007. *Caenorhabditis elegans* integrates the signals of butanone and food to enhance chemotaxis to butanone. J. Neurosci. 27:741–750.

Trivers, R. 1972. Parental investment and sexual selection. In B. Campbell, ed. Sexual Selection and the Descent of Man, 1871–1971. Aldine-Atherton, Chicago, pp. 136–179.

Troemel, E. R., B. E. Kimmel, and C. I. Bargmann. 1997. Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in *C. elegans*. Cell 91:161–169.

Tsui, D., and D. van der Kooy. 2008. Serotonin mediates a learned increase in attraction to high concentrations of benzaldehyde in aged *C. elegans*. Learn Mem. 15:844–855.

Williams, G. C. 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution 11:398–411.

Zwoinska, M., M. I. Lind, and A. A. Maklakov. 2014. Sexual conflict: male control of female longevity. Curr. Biol. 24:R196–R198.

Zwoinska, M. K., N. Kolm, and A. A. Maklakov. 2013. Sex differences in cognitive ageing: testing predictions derived from life-history theory in a dioecious nematode. Exp. Gerontol. 48:1469–1472.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Schematic illustration of the selection procedure.

Figure S2. Schematic illustration of learning assays used in Experiment 1 and 2.

Figure S3. Performance in the selection protocol (Experiment 1). (A) and (B) Naive chemotaxis index; (C) and (D) postconditioning chemotaxis index; (E) and (F) learning index calculated as a difference between post– and naive chemotaxis indices. Left column females, right column males. Data (means ± SEM) represent raw values.

Figure S4. Performance in the original protocol (Experiment 2). (A) and (B) Naive chemotaxis index; (C) and (D) postconditioning chemotaxis index; (E) and (F) learning index calculated as a difference between post– and naive chemotaxis indices. Left column females, right column males. Data (means ± SEM) represent raw values.

Figure S5. Male (B) and female (A) reproductive performance measured as the number of L4 larvae produced. Data (means ± SEM) represent raw values.

Figure S6. Locomotory activity or females (A) and males (B) from upward– (dashed line, triangles) and downward-selected (solid line, circles) lines. Data (means ± SEM) represent raw values.