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Short report

# Sex differences in cognitive ageing: Testing predictions derived from life-history theory in a dioecious nematode

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ABSTRACT

Life-history theory maintains that organisms allocate limited resources to different traits to maximize fitness. Learning ability and memory are costly and known to trade-off with longevity in invertebrates. However, since the relationship between longevity and fitness often differs between the sexes, it is likely that sexes will differentially resolve the trade-off between learning and longevity. We used an established associative learning paradigm in the dioecious nematode *Caenorhabditis remanei*, which is sexually dimorphic for lifespan, to study age-related learning ability in males and females. In particular, we tested the hypothesis that females (the shorter-lived sex) show higher learning ability than males early in life but senesce faster. Indeed, young females outperformed young males in learning a novel association between an odour (butanone) and food (bacteria). However, while learning ability and offspring production declined rapidly with age in females, males maintained high levels of these traits until mid-age. These results not only demonstrate sexual dimorphism in age-related learning ability but also suggest that it conforms to predictions derived from the life-history theory.



## 1. Introduction

Learning ability is a key behavioural trait that affects survival and reproduction of both sexes but there is mounting evidence that learning and memory are costly in vertebrates and invertebrates alike (Dukas, 1998; Kotrschal et al., 2013; Mery and Kawecki, 2003; Snell-Rood et al., 2011). Specifically, learning ability has been shown to trade-off with longevity (Burger et al., 2008; Lagasse et al., 2012; Mery and Kawecki, 2004; Plaçais and Preat, 2013) and reproduction (Snell-Rood et al., 2011). The most striking example of a cost of learning is a symmetrical evolutionary trade-off between learning ability and longevity in the fruit fly *Drosophila melanogaster* (Burger et al., 2008). It was found that experimental populations selected for improved learning suffered a considerable reduction in longevity, while populations selected for increased longevity suffered a massive reduction in learning ability (Burger et al., 2008). Furthermore, learning and memory were also shown to incur constitutive fitness costs in terms of delayed reproduction and lower fecundity in butterflies (Snell-Rood et al., 2011), and reduced larval competitive ability in *D. melanogaster* (Mery and Kawecki, 2003).

Because males and females often resolve their survival vs. reproduction trade-offs differently (Bonduriansky et al., 2008; Maklakov and Lummaa, 2013; Trivers, 1972; Williams, 1957), it is likely that the trade-off between survival and learning will also be resolved differently between the sexes. Applying the logic of a symmetrical evolutionary trade-off (Burger et al., 2008), increased sex-specific investment in learning ability should result in reduced longevity in a given sex. However, the longer-lived sex would experience stronger selection late in life because of a larger number of surviving individuals in older ages (Williams, 1957) such that it should maintain its level of performance for a longer period of time and senesce slower than the shorter-lived sex. This basic pattern can be modified when fitness costs and benefits of learning differ between the sexes.

Despite the evidence that learning ability is likely to trade-off with longevity and the widespread occurrence of sexual dimorphism in lifespan across taxa (Clutton-Brock and Isvaran, 2007), little is known about sex differences in age-related learning across species and whether such differences are in line with predictions derived from life-history theory. This is unfortunate because there is broad cross-disciplinary interest in cognitive differences between the sexes, as well as in sex-specific ageing. Animal models that are accessible for experimental manipulation may prove particularly valuable in linking sexual dimorphism in major life-history traits to sex differences in life-long learning performance.

Here, we used a positive associative learning paradigm in the dioecious nematode worm *Caenorhabditis remanei*, which exhibits sexual dimorphism in longevity (McCulloch and Gems, 2003) and reproductive ageing, to test whether sexes differ in age-related learning performance. Specifically, we tested whether the shorter-lived sex (females in *C. remanei*) exhibits improved learning ability early in life, and whether

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the longer-lived sex (males) shows slower ageing in learning. *Caenorhabditis* nematodes can learn in associative and non-associative paradigms, and form short- and long-term memories. Since learning and memory are known to be actively regulated by highly-conserved longevity pathways in these animals, they emerge as a convenient system for the study of trade-offs between cognitive and life-history traits (Kauffman et al., 2010; Stein and Murphy, 2012).

## 2. Materials and methods

### 2.1. Worm strain and culture

We used an outbred SP8 strain of *C. remanei*, provided by N. Timmermeyer from the Department of Biology at Tuebingen University, Germany. This strain harbours substantial amounts of genetic variation for fitness and life-history traits (Chen and Maklakov, 2012). Worm cultures were recovered from freezing and maintained under standard cultivation conditions (Stiernagle, 2006) for 2–3 generations before being used in experiments. Age-synchronized populations were obtained by isolating eggs through a hypochlorite treatment (Stiernagle, 2006). From the first day of adulthood onwards, animals were transferred to new plates every day to prevent mixing adults with progeny.

### 2.2. Longevity and reproduction assays

For the longevity assay, virgin worms (L4 larvae stage) of the focal sex were placed together with the corresponding number of virgin worms of the opposite sex (10 worms of the focal sex per plate, n = 4 plates for each sex). The sex ratio was adjusted throughout the study period. In the female reproductive assay (n = 13 females), a virgin female was placed with a single male for about 24 h. Each day a female was transferred to a new plate with a fresh male. The male reproductive assay (n = 12 males) was conducted by allowing a single male to mate with 8 virgin females for 3 h. After this time the male was removed and females were allowed to lay eggs for another 3 h. Two days later the progeny, by this time mainly in the L4 stage, were counted.

### 2.3. Behavioural assays

Behavioural assays were conducted at days 2, 5 or 8 of adulthood, which roughly corresponds to young, middle-aged and elderly adults in this population (Chen and Maklakov, 2012), and followed the protocol from Kauffman et al. (2010) (for details see Electronic Supplementary Material). Prior to testing, males and females were separated and left overnight on Petri plates with nematode growth medium (NGM) and bacterial food source (*Escherichia coli* OP50). Three behavioural responses were quantified: motility, naïve chemotaxis to butanone, and post-conditioning chemotaxis to butanone. For all assays, animals were placed on 92 mm NGM plates (no food) on the place of origin and allowed to move freely for 1 h. After this time, assays were terminated by inverting the plate over a few drops of chloroform and then worms were hand-counted. The number of worms per assay ranged from 50 to 151. Motility was quantified by calculating the motility index (MI) according to the following formula: $MI = n_{\text{left the place of origin}} / (n_{\text{total on a plate}} - n_{\text{dead on the place of origin}})$ (we subtracted all worms that have been accidentally killed or injured during testing, this was not, however, a big number), where n represents the number of worms. Naïve chemotaxis to butanone was estimated by placing naïve worms on the place of origin at equal distance from 10% butanone and ethanol (control) odour patches at the opposite sides of the plate (Fig. S1).1.5 μl

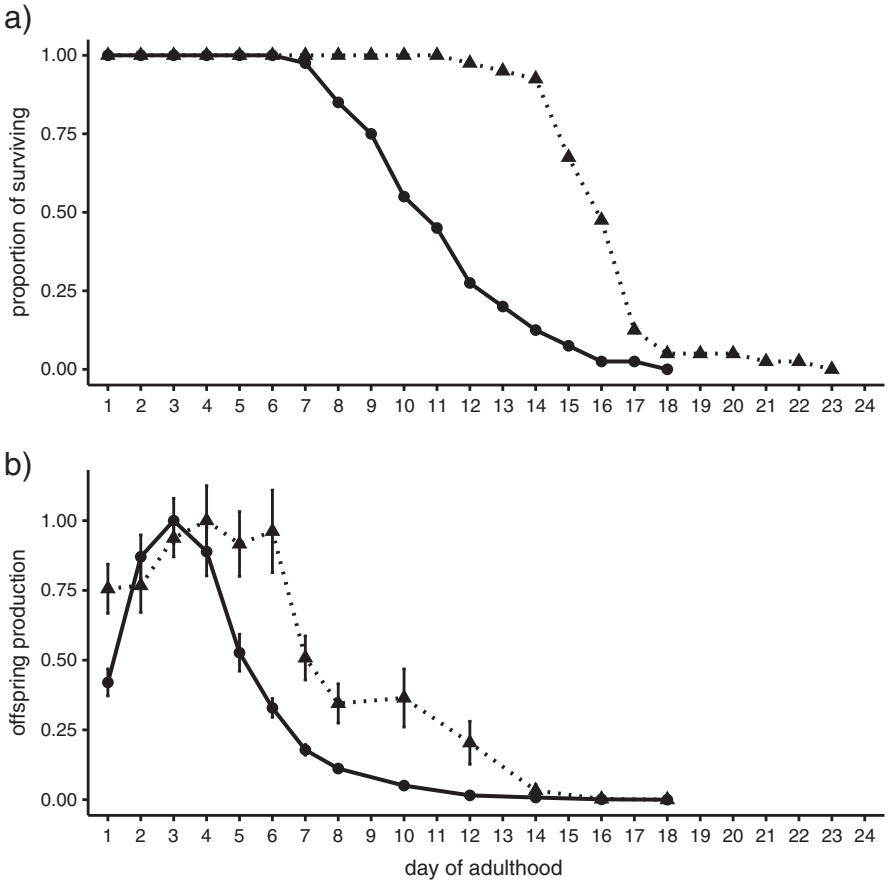


**Fig. 1.** (a) Survival and (b) reproduction of males and females *C. remanei* from the first day of adulthood onwards. For illustrative purpose offspring production is presented as the mean of the number of larvae per individual for a given day divided by the highest mean of larvae produced per day; ±SEM. Solid lines, circles — females and dotted lines, triangles — males.

of an anaesthetic-sodium azide, $NaN_3$, was placed on the odour spots before adding butanone or ethanol to immobilize worms that reach them. As in the motility assay, worms moved freely for 1 h. Naïve chemotaxis index was calculated with the following formula: Chemotaxis Index (CI) = ($n_{attractant\ spot} - n_{control\ spot}$)/($n_{total\ on\ a\ plate} - n_{dead\ on\ the\ place\ of\ origin}$). The learning procedure involved 1 h of starvation in M9 buffer, followed by 1 h of conditioning. Conditioning procedure was performed on 60 mm NGM plates in the presence of food — 0.5 ml of *E. coli* OP50 (unconditioned stimulus) and 2 μl of butanone streaked on the lid of the plate (conditioned stimulus). After 1 h, animals were washed with M9 buffer and tested for post-conditioning chemotaxis to butanone. Post-conditioning chemotaxis assay was performed the same way as naïve chemotaxis assay. To quantify learning we used the following formula: Learning Index (LI) = $CI_{post\text{-}conditioning} - CI_{naïve}$. All animals were used only once.

### 2.4. Data analysis

Statistical analyses were performed in JMP 10 using general linear mixed models with age, age$^2$, sex and their interactions as the fixed factors and trial as the random factor. Learning performance was assessed with two different models: in the first model, we followed a standard approach and used learning index as a response variable. Learning index was calculated by subtracting naïve chemotaxis index from post-conditioning chemotaxis index. In the second model, we used post-conditioning chemotaxis index as a response variable and naïve chemotaxis index as a covariate. To select the final model we used backward elimination procedure and corrected Akaike Information Criterion. Survival curves were analysed with the log-rank test.

## 3. Results

Survival differed between the sexes (log-rank test, $\chi^2 = 52.2133$, df = 1, $p < 0.0001$) with males living substantially longer than females (Fig. 1a). Reproductive ageing was also sexually dimorphic (age × sex $F_{1,\ 372.4} = 8.0695$, $p = 0.0047$ and age$^2$ × sex $F_{1,\ 372.4} = 23.1558$, $p < 0.0001$) (Table S1) as males reached their reproductive peak later than females and maintained high offspring production for longer (Fig. 1b). Age ($F_{1,\ 27.47} = 17.9419$, $p = 0.0002$) and sex ($F_{1,\ 23.96} = 14.8641$, $p = 0.0008$) had a strong effect on motility during the first 8 days of adulthood, but their interaction was not significant ($F_{1,\ 23.96} = 4.1319$, $p = 0.0533$) (Table S2, Fig. 2a). We found a non-linear relationship between age and sex in naïve chemotaxis, as demonstrated by the significant age$^2$ × sex interaction ($F_{1,\ 53.6} = 12.5503$, $p = 0.0008$), driven by the difference in performance of 5-day old worms (Table S3, Fig. 2b). When learning performance was assessed using learning index as a response variable, we found significant effects of age, sex and age$^2$ × sex interaction, the latter driven by decreased male performance among 2-day old worms and decreased female performance when worms were 5-days of age (Table 1, Fig. 2d). On the other hand, when the model with post-conditioning chemotaxis as a response variable and naïve chemotaxis as a covariate was employed, only the main effects of age and the age × sex interaction were significant, reflecting reduced male performance among 2-day old worms (Table 1).

## 4. Discussion

Learning ability can be a major contributor to individual fitness, yet only recently there has been considerable effort to integrate learning and memory into life-history framework (Burger et al., 2008; Dukas,

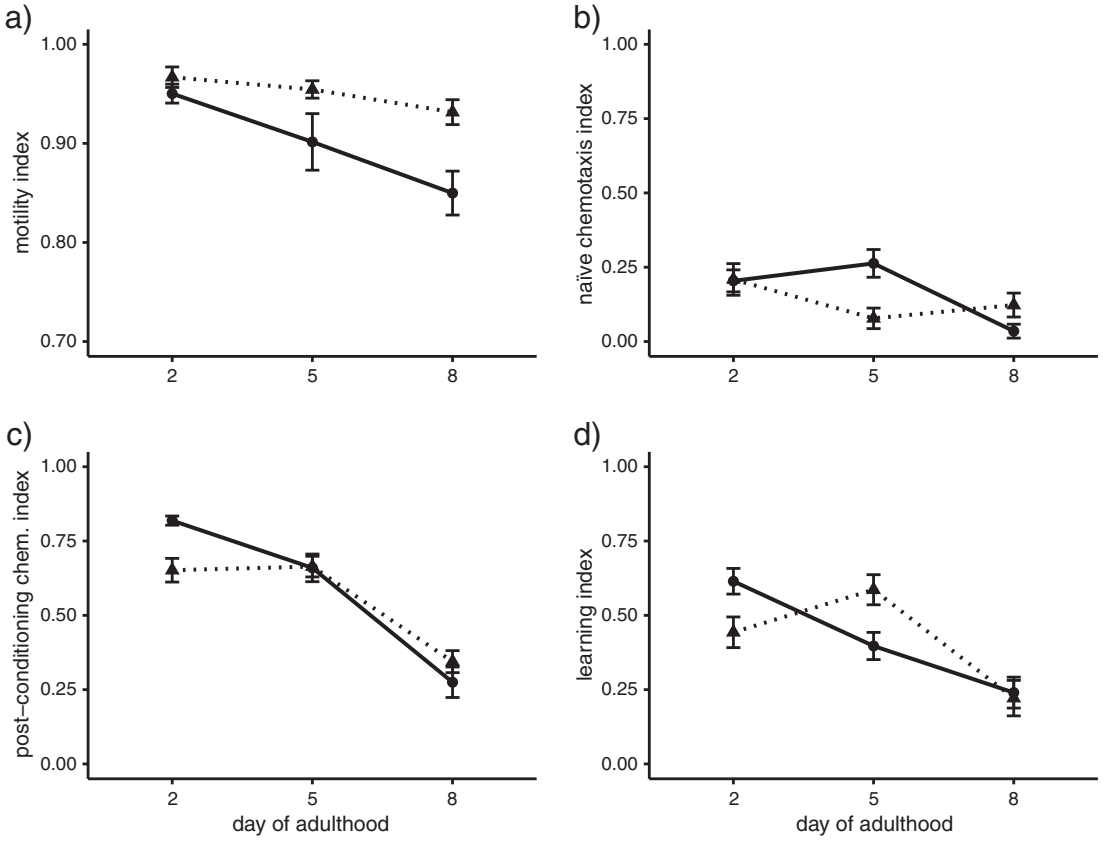


**Fig. 2.** Behavioural responses of *C. remanei*: (a) motility, MI = $n_{left\ the\ place\ of\ origin}$/($n_{total\ on\ a\ plate} - n_{dead\ on\ the\ place\ of\ origin}$), (b) naïve chemotaxis to 10% butanone, (c) post-conditioning chemotaxis to 10% butanone. Chemotaxis index (CI) for naïve and post-conditioning chemotaxis was calculated as CI = ($n_{attractant\ spot} - n_{control\ spot}$)/($n_{total\ on\ a\ plate} - n_{dead\ on\ the\ place\ of\ origin}$). (d) Learning index as calculated by subtracting $CI_{naïve}$ from $CI_{post\text{-}conditioning}$. N = 6 for motility assays and N = 12 for naïve and post-conditioning chemotaxis assays; ±SEM. Solid lines, circles — females and dotted lines, triangles — males.

**Table 1**
General linear mixed models of the effects of age, sex and naïve chemotaxis on post-conditioning chemotaxis to butanone and of age and sex on learning index. The columns provide degrees of freedom (DF), F-ratios and *P*-values for fixed effects and lower and upper 95% confidence limits and total percent explained variance for random effects.

| Model 1: Response post-conditioning chemotaxis | | | |
|---|---|---|---|
| Fixed effects | DF | F | *p*-Value |
| Age | 1.6169 | 98.9938 | <0.0001 |
| Sex | 1.5346 | 0.4555 | 0.5026 |
| Age × sex | 1.5212 | 13.1828 | 0.0006 |
| Age$^2$ | 1.5767 | 17.2133 | 0.0001 |
| Age$^2$ × sex | 1.5364 | 2.2510 | 0.1394 |
| Naïve chemotaxis | 1.5972 | 1.1792 | 0.2819 |
| Naïve chemotaxis$^2$ | 1.6188 | 0.3447 | 0.5593 |
| Random effects | 95% lower | 95% upper | % |
| Trial | −0.001623 | 0.0177013 | 41.054 |
| Model 2: Response learning index | | | |
| Fixed effects | DF | F | *p*-Value |
| Age | 1.6427 | 28.0580 | <0.0001 |
| Sex | 1.5203 | 9.0066 | 0.0041 |
| Age × sex | 1.5203 | 2.9506 | 0.0918 |
| Age$^2$ | 1.6073 | 6.5028 | 0.0133 |
| Age$^2$ × sex | 1.5203 | 13.5586 | 0.0006 |
| Random effects | 95% lower | 95% upper | % |
| Trial | −0.004638 | 0.0249154 | 29.819 |

1998; Kotrschal et al., 2013; Lagasse et al., 2012; Mery and Kawecki, 2003). Sex emerges as an important factor to be considered in such studies as males and females have divergent reproductive strategies, and consequently different life-histories (Trivers, 1972). We found that the dioecious nematode *C. remanei* shows strong sexual dimorphism in lifespan, supporting a previous study (McCulloch and Gems, 2003), and in reproductive ageing, as males lived longer than females and their reproductive performance increased and peaked at later ages than in females. Most notably, we showed that age-related learning ability also differed between the sexes. Although the two models used to asses learning disagreed on the exact shape of the curve, they both showed that while females outperformed males early in life in the associative learning task, their learning performance declined by middle-age. Males, on the other hand, maintained their performance for longer and started to deteriorate later in life than females. The learning index is the standard way to examine learning performance in nematode studies, nevertheless, we advocate combining it with an additional approach with post-conditioning chemotaxis as a response variable and naïve chemotaxis as a covariate. This is because the sex-specific difference in naïve chemotaxis at different ages might obscure simple interpretation of the learning index.

Despite the evidence for the evolutionary trade-off between learning ability and longevity in *Drosophila* studies (Burger et al., 2008; Lagasse et al., 2012), its importance across species remains unknown. Building upon life-history theory we derived predictions that allowed us to confront the hypothesis of the evolutionary sex-specific trade-off between learning and longevity with experimental data. In accordance with these predictions we found that the shorter-lived sex – females – performed better in a learning task early in life but deteriorated faster than the longer-lived sex — males. Further investigations are, however, needed to establish if learning has sex-specific costs and benefits in our study species, as it may also influence life-long learning performance.

Although the sex-specific pattern of cognitive decline we documented can likely be shaped by the learning-longevity trade-off, there are alternative explanations to consider. For instance, the pattern we found might be specific to the particular learning protocol. Noteworthy, however, another study found that *C. elegans* hermaphrodites (which are morphologically equivalent to females and live shorter than males) outperformed males early in life in making a novel association between salt and starvation (Vellai et al., 2006). Secondly, the motivational value of food (i.e. bacteria) may differ between the sexes and across ages. While we cannot address this issue directly, we note that attraction to a bacterial metabolite, benzaldehyde, increases in *C. elegans* and both sexes of *C. remanei* as they age (Tsui and van der Kooy, 2008; Zwoinska, personal observation).

In conclusion, we demonstrated that age-related learning differs between the sexes, and that it corresponds with sexual dimorphism in life-history. We argue that evolution of sex differences in cognitive abilities is likely to be shaped by sex-specific life-histories and also suggest that dioecious nematodes provide a powerful model system for future research in this area.

## Conflict of interest

The authors have no conflicts of interests.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.exger.2013.09.008.

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