

## Secondary compounds from exotic tree plantations change female mating preferences in the palmate newt (*Lissotriton helveticus*)

M. IGLESIAS-CARRASCO\* † , M. L. HEAD ‡, M. D. JENNIONS ‡ § & C. CABIDO †

\*Department of Evolutionary Ecology, National Museum of Natural Sciences, Spanish National Research Council (CSIC), Madrid, Spain

†Department of Herpetology, Aranzadi Science Society, Donostia-San Sebastián, Spain

‡Division of Evolution, Ecology and Genetics, Research School of Biology, Australian National University, Canberra, ACT, Australia

§Wissenschaftskolleg zu Berlin, Berlin, Germany

### Keywords:

anthropogenic disturbance;  
female choice;  
*Lissotriton helveticus*;  
sexual selection;  
Urodeles.

### Abstract

Selection can favour phenotypic plasticity in mate choice in response to environmental factors that alter the costs and benefits of being choosy, or of choosing specific mates. Human-induced environmental change could alter sexual selection by affecting the costs of mate choice, or by impairing the ability of individuals to identify preferred mates. For example, variation in mate choice could be driven by environmentally induced differences in body condition (e.g. health) that change the cost of choosiness, or by environmental effects on the ability to detect or discriminate sexual signals. We teased apart these possibilities experimentally, by comparing female mate choice in the palmate newt *Lissotriton helveticus* between environments that mimic water from either native oak forests or exotic eucalypt plantations. In laboratory two-choice mate trials in clean water, females with prolonged exposure (21 days) to waterborne chemicals leached from eucalypt leaves did not preferentially associate with the male with a stronger immune response, but females exposed to water with chemicals from oak leaves did. In contrast, female choice was unaffected by the immediate presence or absence of eucalypt leachates during mate choice (using only females previously held in oak-treated water). The habitat-related change in female choice we observed is likely to be driven by effects of eucalypt leachates on female physiology, rather than immediate inhibition of pheromone transmission or blocking of pheromone reception.

### Introduction

If mating nonrandomly confers benefits, then mate choice can lead to sexual selection for costly but attractive traits. Mate choice is, however, itself costly due to the additional time, energy and predation risks that arise when assessing and then rejecting potential mates (Kokko *et al.*, 2015). In some species, an increase in the costs of mate choice is associated with a reduction in choosiness by both females (Hunt *et al.*, 2005; Head

*et al.*, 2010; Tomkins *et al.*, 2016) and males (Wong & Jennions, 2003). Ultimately, mate choice is only maintained by selection if the benefits of expressing mating preferences exceed the costs of choosiness. As with most life-history traits (review: DeWitt *et al.*, 1998), there are therefore circumstances where selection will favour phenotypic plasticity in mating preferences in response to environmental factors that alter the costs and benefits of mate choice.

Increasingly, species are being confronted with novel environments due to human habitat disturbance. These new environments could change the intensity and direction of sexual selection (Candolin *et al.*, 2007). One factor that could drive these changes is the costs of mate choice, such that previously adaptive preferences or choosiness (for the distinction, see Jennions & Petrie,

Correspondence: Maider Iglesias-Carrasco, Department of Evolutionary Ecology, Museo Nacional de Ciencias Naturales (CSIC), José Gutiérrez Abascal, 2. 28006 Madrid, Spain.  
Tel.: +34 943 466 142; fax: +34 91 564 5078;  
e-mail: miglesias15@gmail.com

1997) become maladaptive (i.e. greater costs favour reduced choosiness; Head *et al.*, 2010). This could have long-term consequences because sexual selection can affect the viability and rate of adaptation of populations to new conditions (review: Candolin & Heuschele, 2008; Power & Holman, 2015).

Many studies report differences among populations in female mating preferences (e.g. Boughman, 2001; Kwiatkowski & Sullivan, 2002), but relatively few studies relate these differences to habitat-dependent effects (Maan *et al.*, 2006; Rafferty & Boughman, 2006; Heuschele *et al.*, 2009). Even fewer studies take the final step of conducting experiments to directly test which aspects of the habitat drive differences in mate choice. There are therefore few studies that document habitat variation in female mate choice and then provide a functional account for its occurrence. Here, we pursue this goal by comparing female mate choice in European palmate newts (*Lissotriton helveticus* Razoumowsky, 1789), a common urodele in Western Europe, in two well-defined environments: native oak forest and a recently established exotic eucalypt plantation used by our study species. Eucalypt plantations are anthropogenic habitats, created in the 1950s in the study area. Eucalypt plantations are characterized by the release of secondary compounds into waterways, such as polyphenols and tannins. These compounds can be toxic to fish (Steinberg *et al.*, 2006), aquatic invertebrates (Larrañaga *et al.*, 2009b) and amphibians (Iglesias-Carrasco *et al.*, 2016) and can affect growth (Canhoto & Laranjeira, 2007), development (Martin & Blossey, 2013), reproduction (Morrongiello *et al.*, 2011) and immune response (Iglesias-Carrasco *et al.*, 2016) of aquatic taxa. Experiments further show that these chemicals also affect the behaviour of Australian species that have a long history of co-existence with *Eucalyptus* (Morrongiello *et al.*, 2011). However, how plantations affect the sexual behaviour of species, such as many in Europe, that have only recently come into contact with the chemicals released by eucalypts has not been explored.

Variation in female choice could result from physiological differences that arise due to environmental effects (e.g. high parasite loads reducing body condition); or it could arise because environmental factors have an immediate, direct effect on the ability of females to discriminate between potential mates. To tease apart these two hypotheses, we performed two experiments using two-choice mate trials in which each female was allowed to choose between two males that differed in their immune response. We based our experiments on this male physiological trait because it is condition dependent and positively correlated with the expression of a number of other sexually selected characters (Iglesias-Carrasco *et al.*, 2016). In the first experiment, we compared female mate choice in clean water after females had prolonged (21-day) exposure to

waterborne chemicals from either native oak or exotic eucalypt leaves. Female newts exposed to chemicals released by eucalypt plantations might allocate stored resources to combat toxicity, so that other life-history traits are adversely affected. We therefore predicted that females exposed to eucalypt chemicals would be less discriminating, as is generally the case for females under stress (Hunt *et al.*, 2005). Second, we took females that had only been exposed to waterborne chemicals from native oak (21 days) and compared their mate choice in water that contained waterborne chemicals from either oak or eucalypt leaves. Female newts use both visual signals and pheromones to choose mates (Houck, 2009), so a decline in water quality due to toxic substances released by eucalypt leaves might impair chemical communication between the sexes (Park *et al.*, 2001). We therefore predicted that females tested in water containing eucalypt chemicals, when deprived of visual cues, would be less capable of discriminating between males. This could occur because waterborne eucalypt chemicals reduce the efficiency with which pheromones are transmitted, or because they lower the ability of females to detect pheromones (i.e. alter their sensory capabilities). From these experiments, we use trials where female mate choice occurred under natural conditions (i.e. native oak water) to establish a baseline of female choice for male ability to respond to an immune challenge. Under natural conditions, females are expected to choose males with a stronger immune response.

## Materials and methods

We conducted two experiments. First, we exposed females to eucalypt-infused water for a period of 21 days in mesocosms (hereafter called 'prolonged exposure' to differentiate from the 'immediate' or 'short-term exposure' in Experiment 2) (*see Experiment 1*) and then tested their mate choice. Second, we tested whether mate choice differed when females maintained in native oak-infused water were tested in water from their natural habitat or in water that was eucalypt-infused (*see Experiment 2*). We conducted dichotomous choice tests where females were able to choose between two males. We measured male immune responses (following a phytohaemagglutinin (PHA) injection assay, *see below*) as an index of mate health. We ensured that male pairs covered a range of differences in PHA immune responses (from 0.60 to 0.02), so females had to choose between pairs of males that varied in how much they differed in immune response (mean difference and SE for each treatment: Experiment 1: eucalypt  $0.215 \pm 0.149$ ; oak  $0.203 \pm 0.139$ . Experiment 2: eucalypt =  $0.148 \pm 0.07$ ; oak =  $0.164 \pm 0.09$ ). We expected that the more the males differed, the stronger the choice would be for the male with a greater immune response. All the males were adults

and there was no difference in body size between treatments (mean size (mm) and SE: Experiment 1: eucalypt =  $35.78 \pm 2.01$ ; oak =  $35.65 \pm 1.78$ . Experiment 2: eucalypt =  $35.44 \pm 1.73$ ; oak =  $35.62 \pm 1.61$ ).

### Female's maintenance

Palmate newts in Spain breed in spring and summer (February to June) in a range of waterbodies, from ponds to lakes, and occur in a variety of habitats, from intensively managed agricultural lands to natural forests. In forested areas, newts are far more common in native forests than in eucalypt plantations, but they are found in both habitats. On 7th and 8th March 2014, in the middle of the mating season, we captured by dip-netting 182 adult female newts (7th March Experiment 1:  $n = 120$ ; 8th March Experiment 2:  $n = 62$ ) from ponds in native deciduous forests (mainly oak *Quercus robur*). We sampled three ponds within a habitat patch of 1.2 km<sup>2</sup>. Given that newts move between ponds (up to several km apart, Montori & Herrero, 2004), the collected individuals can be considered to come from a single population. For Experiment 2, it would also have been interesting to test whether there is adaptation or acclimation to eucalypt leachates by testing females from eucalypt plantations. However, females were rare in ponds under eucalypt, and the sample size we obtained was insufficient ( $n = 18$ ) to conduct the experiment. After collection, female newts were brought back to the laboratory at Aranzadi Science Society and housed in mesocosms. To simulate natural pond conditions, we used 470-L outdoor mesocosms containing 200 L of mineral water and 5 L of natural pond water containing zooplankton and algae. We set up three mesocosms per habitat type (three oak replicates and three eucalypt replicates for Experiment 1; three oak replicates for Experiment 2). In each mesocosm, we added 100 g of dried leaves (see Maerz *et al.*, 2005 for dose rates), either eucalypt (treatment) or oak (control). To prevent the entry of predators, we covered each mesocosm with mesh. After 48 h, we placed 20 females into each mesocosm. We added 5 g of blood worms daily. Females were kept in the mesocosms for 21 days, which is a standard period for behavioural ecotoxicology studies (Sparling *et al.*, 2010). This 21-day period has been shown to be ecologically meaningful for amphibians, as the exposure to plant leachates for 3 weeks increases mortality and reduces growth of amphibian larvae (Maerz *et al.*, 2005; Watling *et al.*, 2011), and it reduces the immune response of adult newts (Iglesias-Carrasco *et al.*, 2016).

### Male immune response

We used male immune function as a likely predictor of female mate choice for two reasons. First, it is better than measuring any given sexually selected trait because these traits often show phenotypic trade-offs.

In contrast, immune function gives an overall estimate of health that is correlated with key traits assessed by females when choosing mates in both habitats (for field data, see Iglesias-Carrasco *et al.*, 2016). Second, measuring immune responses on the large number of males we tested is quicker than measuring numerous sexually selected traits. This allowed us to use males shortly after capture from the wild, rather than having to keep them in captivity for a long period prior to testing.

We captured 240 adult male newts in native oak forest patches 3 days prior to behavioural trials. We measured their immune function as inflammation following a PHA injection assay ('PHA immune response'). PHA immune response is a reliable measure of T-cell-dependent immunocompetence *in vivo* (Lochmiller *et al.*, 1993), which has been used and validated in several studies, including ones on amphibians (Clulow *et al.*, 2015; Murillo-Rincón *et al.*, 2017). We measured the thickness at the base of the tail 5 mm from the vent with a pressure-sensitive spessimeter (+0.01 mm). Immediately afterwards, we injected 0.01 mg of PHA dissolved in 0.01 mL of phosphate-buffered saline (PBS). After 24 h, we again measured tail thickness and calculated the difference between the pre- and post-injection measurements (i.e. inflammation).

### Experiment 1: Prolonged exposure to eucalypt leaf litter and female mate choice

#### Behavioural trials

We removed females from mesocosms with an aquarium net 21 days after first housing them in the mesocosms. We placed them individually in  $36 \times 42$  cm aquaria with 10 L of mineral water, with a natural photoperiod and constant temperature of 12 °C. Females were allowed 1 h to acclimate to these conditions, before two males were added. To ensure that female choice depended on chemical cues, males were placed in an opaque 1-L cup ( $7 \times 7 \times 20$  cm) pierced with numerous small holes to prevent tactile and visual stimuli, but allow water exchange. A cup was placed at each end of the aquarium and females could freely move between them. We allowed 20 min for the newts to acclimate before observations began. Then, every 10 min for 150 min, we noted whether the female was < 5 cm from a cup. Previous studies have shown that the relative time spent with males is a good predictor of female mate choice (Amundsen & Forsgren, 2001; Milinski *et al.*, 2005; Heuschele *et al.*, 2009). Moreover, using a subset of females, we confirmed that females mate with the male with whom they preferentially associated ( $n = 18/20$ ; binomial test,  $P < 0.001$ ). Placement of aquaria was randomized to avoid potential environmental gradients within the laboratory confounding our treatments. All the trials were conducted on the same day and began at 9 pm (i.e. after dark) to

ensure that females were active and searching for mates (based on activity patterns in a similar species, Griffiths (1985) and confirmed in our study species by MIC). The behavioural trials were made in darkness at a constant temperature of 12 °C, mirroring the air temperature in the field on the night of the experiment. MIC collected all data blind to both the females' treatment and the males' PHA immune response. All animals remained healthy during the trials and were eventually re-released at the capture sites.

### Experiment 2: The immediate effect of eucalypt leaf litter on female mate choice

To test the immediate effects on female mate choice of the presence of secondary compounds from eucalypt leaf litter, we conducted a second experiment where *all* females ( $n = 62$ ) were housed in mesocosms containing oak-treated water for 21 days. We then quantified female choice for males that differed in their immune response as in Experiment 1 (see above), except that the test aquarium now contained either 10 L of oak-infused water (control) or eucalypt-infused water ( $n = 31, 31$ ). We opted for the preparation of new extracts and did not directly use the water from the mesocosms from Experiment 1 to eliminate other factors (e.g. female and food odour) that might affect the behaviour of our study animals. Infusions were prepared 48 h prior to the trials by adding 5 g of dried leaves to the water (dose rates, Maerz *et al.*, 2005). Previous studies looking at leachate composition of water show that 48 h is enough time for plant secondary compounds to be leached into the water (Maerz *et al.*, 2005; Watling *et al.*, 2011). Leaves were strained with cheesecloth from the leachate solution to remove solid material immediately before the choice trial. After 1 h of acclimation, a pair of males was added to the aquarium and female choice was recorded.

### Statistical analyses

In our data set, tail inflammation was correlated with body size ( $R^2 = 0.16$ ,  $P < 0.001$ ). However, given that the paired males were similar in size in both treatments and in both experiments (mean difference in size (mm)  $\pm$  SE between the male with the stronger and the weaker immune response: Experiment 1 eucalypt:  $-0.15 \pm 2.67$ ; oak:  $0.16 \pm 2.35$ . Experiment 2 eucalypt:  $1.41 \pm 3.01$ ; oak:  $0.13 \pm 2.66$ ) and that preliminary analyses showed no effect of male size difference on female preferences ( $P$ -values of the model for both experiments  $> 0.27$ ), we chose not to control for body size in our analyses (Freckleton, 2001). Note however, that controlling for body size did not alter our results.

First, we tested with  $\chi^2$  tests whether there was a difference in female interest in males between the two

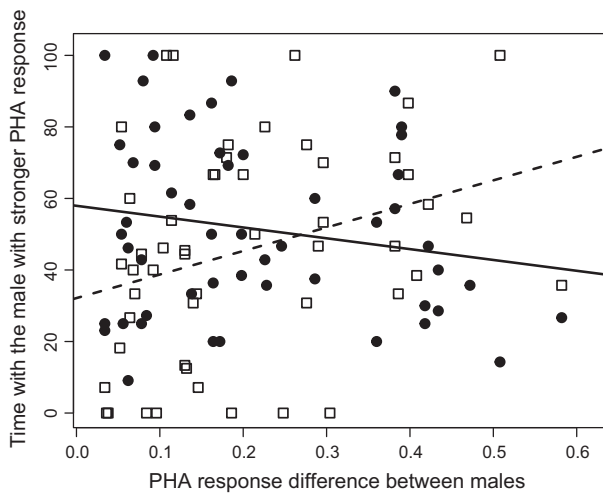
habitats. We compared the proportion of females from each treatment that showed no interest in males (i.e. hung at the surface and did not explore the aquaria) (Experiment 1:  $n = 16/120$ ; Experiment 2:  $n = 5/62$ ).

For the rest of the analyses, we discarded trials where the females showed no interest in males. The final sample sizes were 53 eucalypt- and 51 oak-treatment females in Experiment 1 and 28 eucalypt- and 29 oak-treatment females in Experiment 2. We initially analysed our data with a generalized linear mixed model (GLMM) with a binomial error distribution, but the data were highly overdispersed (the scaling parameter was 4.26 in Experiment 1 and 1.57 in Experiment 2 for models that included the interaction term). A quasibinomial error distribution cannot be used when models include random effects, so we opted to analyse the proportion of observations a female spent with the higher-quality male (i.e. male with the stronger immune response) using linear mixed models (LMM) for both experiments. We checked and confirmed that the residuals of all models met the assumptions of normality, linearity and homoscedasticity (Fig. S1 and S2). First, to test whether the time females spent associating with males differed among treatments, we ran a model for each experiment that included the treatment as a fixed term and the mesocosms replicate as a random factor. Then, for each experiment, we ran separate models that included the water treatment, the difference in PHA immune response between the pair of males and the interaction between these variables as fixed terms. We included mesocosm replicate identity as a random factor. All the analyses were run in R 3.2.2. The two key statistical tests ask: (i) Is there a positive effect of the magnitude of the difference in male PHA immune response on the proportion of time females spent with the male with the stronger immune response? If so, this indicates that females spent relatively more time with the male with a greater immune response when the two males are more readily distinguished, and (ii) Is there an interaction between water treatment and the difference in PHA immune response that affects the proportion of time spent with the male with the stronger immune response? If so, this indicates that ability of females to discriminate between males that differ in their PHA immune response depends on the water treatment.

## Results

### Experiment 1: Prolonged exposure to eucalypt leaf litter and female mate choice

There was no effect of the water treatment on the level of female interest in males ( $\chi^2 = 0.062$ ,  $P = 0.802$ ). Neither was there an effect of the treatment on the average time that females spent associating with males ( $F_{1,101} = 1.20$ ,  $P = 0.275$ ).



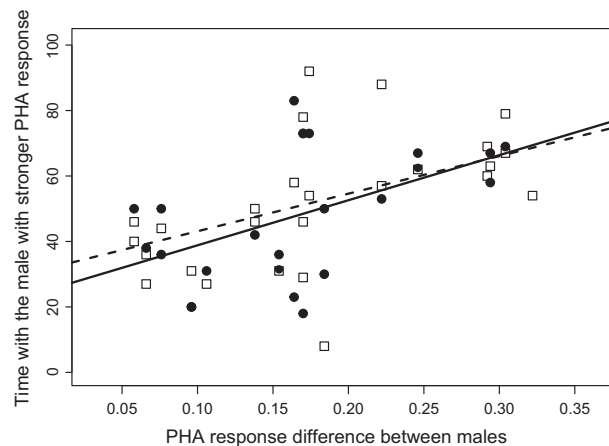
**Fig. 1** The relative time spent near the male with the stronger PHA immune response (% of point samples) by females housed in either eucalypt-treated mesocosms (solid line, black circles) or oak-treated mesocosms (dashed line, white squares). Graph shows the raw data.

However, the water treatment did affect how females responded to the difference in males' PHA immune response (interaction estimate  $\pm$  SE =  $96.10 \pm 36.55$ ,  $t = 2.62$ ,  $P = 0.009$ ). To look at female preference for male PHA immune response within each treatment, we ran separate analyses for each water treatment. Females from oak-treated mesocosms spent significantly more time near the male with the stronger PHA immune response as the difference between the two males increased (estimate  $\pm$  SE =  $65.82 \pm 26.85$ ,  $t = 2.45$ ,  $P = 0.016$ ). In contrast, eucalypt-treated females showed a nonsignificant preference in the opposite direction (estimate  $\pm$  SE =  $-30.18 \pm 24.80$ ,  $t = -1.22$ ,  $P = 0.225$ ) (Fig. 1).

### Experiment 2: The immediate effect of eucalypt leaf litter on female mate choice

Again, we did not find any effect of the water treatment on the level of interest females had in males ( $\chi^2 = 0.065$ ,  $P = 0.797$ ), or in the time females spent associating with males ( $F_{1,48.79} = 0.25$ ,  $P = 0.617$ ).

Contrary to expectation, females tested in oak-infused and eucalypt-infused water behaved similarly when choosing males (estimate  $\pm$  SE =  $-37.30 \pm 61.09$ ,  $t = -0.20$ ,  $P = 0.841$ ). Females spent significantly more time near the male with the stronger PHA immune response as the difference between the two males increased, regardless of the water type they were tested in (oak: estimate  $\pm$  SE =  $111.00 \pm 34.55$ ,  $t = 3.21$ ,  $P = 0.002$ ; eucalypt: estimate  $\pm$  SE =  $148.30 \pm 50.12$ ,  $t = 2.95$ ,  $P = 0.004$ ) (Fig. 2).



**Fig. 2** The relative time spent near the male with the stronger immune response (% of point samples) by females housed in oak-treated water, but tested in either eucalypt-treated water (solid line, black circles) or oak-treated water (dashed line, white squares). Graph shows the raw data.

## Discussion

Both natural and sexual selection can differ between native and human-modified environments (Candolin & Wong, 2012). In two-choice laboratory mating trials, we found that female palmate newts kept and tested in water that mimics their natural habitat prefer males with a stronger immune response to a PHA injection assay. Females exposed to eucalypt-infused water for a prolonged period (21 days) showed reduced levels of mate choice for these males compared to females exposed for the same period to water mimicking their natural habitat. In contrast, when females were initially kept in water mimicking their natural habitat, the immediate presence of eucalypt-infused water during mate choice did not reduce their choice for males with a stronger immune response. We also found that the interest females had in males and the amount of time spent with males by females were similar for the treatments in both experiments. Our results suggest that females generally prefer males with a stronger immune response; female olfactory capacity and the diffusion of pheromones are unaffected by the immediate presence of eucalypt leaf-litter; but that prolonged term exposure to eucalypt leaf-litter may reduce a female's ability to, or interest in, mating with males with a stronger immune response. If these results apply in the wild, the strength of sexual selection on male pheromones will differ between native forests and exotic eucalypt plantations.

### Effect of prolonged exposure to eucalypt water on female mate choice

Females showed weaker mate choice for males with a strong immune response after prolonged exposure to

eucalypt-infused water compared to water that mimicked natural oak forests. Eucalypt trees leach chemical compounds into the water (e.g. polyphenols and tannins), which can adversely affect the diversity of aquatic animals and their investment in life-history traits (Maerz *et al.*, 2005; Larrañaga *et al.*, 2009a; Morrongiello *et al.*, 2013). Here, we show that prolonged exposure to these compounds can also alter behaviours that directly affect sexual selection. Across both experiments, in three of the four treatment groups, females significantly preferred to associate with (and would mate with, see *Methods*) males that had a higher PHA immune response: the relative amount of time spent with the male that had a greater immune response increased when the difference in immune response between the two males was greater. In contrast, female newts that were exposed to eucalypt leachates for 21 days showed no tendency to associate with males with a stronger immune response (Fig. 1). Males with a higher PHA immune response are generally considered to be in better condition, and tend to have larger secondary sexual traits (e.g. Møller *et al.*, 1999; Navarro *et al.*, 2003; Schmid-Hempel, 2003), so this change in mate choice by females that have had prolonged exposure to leachates could be considered a reduction in preference for high-quality males. If this is the case, the lack of mate choice after prolonged exposure to eucalypt leachates might have consequences for long-term population viability of palmate newts inhabiting eucalypt plantations.

It has been suggested that exposure to plant leachates can cause physiological stress. For example, carp extracts can damage fish gills and possibly interfere with respiration (Temminck *et al.*, 1989). Likewise, exposure to eucalypt leachates is known to affect survival and reproduction of some Australian fish species. For instance, chronic exposure to eucalypt leachates negatively affected growth and survival in relation to body size and source population of juvenile pygmy perch (*Nannoperca australis*) (Morrongiello *et al.*, 2013); however, leachate-exposed females of the same species were less likely to reach maturity and reproduce (Morrongiello *et al.*, 2011). Amphibians are also highly susceptible to habitat alteration and the presence of toxic substances in the water (e.g. Watling *et al.*, 2011). For example, the body condition and immune response of both adult and larval amphibians may be reduced by the presence of crops and exotic eucalypt plantations (Brodeur *et al.*, 2011; Iglesias-Carrasco *et al.*, 2016; Iglesias-Carrasco *in prep.*). We hypothesize that the absence of female mate choice for higher-'quality' males after prolonged exposure to eucalypt leachates may be due to changes in female physiology. Previous studies have suggested that female fish exposed to eucalypt leachates have fewer resources available to allocate to reproduction because they expend energy to resist the effects of leachates (Morrongiello *et al.*, 2011). For instance, combating toxins derived from eucalypt leachates might

make females less choosy because they have fewer resources to allocate to mate sampling (Hunt *et al.*, 2005). However, the fact that female association time with males did not differ between the water treatments suggests that female responsiveness or choosiness (when defined as the time invested by a female in making a choice, see Brooks & Endler, 2001) was unaffected. Alternatively, prolonged exposure to toxins might reduce a female's investment into traits that are required to assess mates (e.g. neurological pathways), or it might simply damage a female's sensory apparatus, and thereby lower her ability to discern differences among males. Teasing apart these competing proximate explanations is an interesting avenue for future research.

In a previous study, we showed that male palmate newts in eucalypt plantations have a reduced immune response and smaller sexual characters than those in native oak forests (Iglesias-Carrasco *et al.*, 2016). We suggested that this was due to lower male body condition given the stress of living in eucalypt plantations (Iglesias-Carrasco *et al.*, 2016). However, our current results suggest that reduced expression of male sexual characters could partly reflect weaker sexual selection due to a lack of mate choice (Candolin *et al.*, 2007): females exposed to eucalypt-infused water no longer discriminate between males. An alternative is that the difference in mate choice by females housed in the two water types might represent adaptive plasticity in mating preferences if the traits that signal male fitness differ between habitats. It is generally assumed that mating preferences are adaptive and, in the absence of direct benefits, signal male genetic quality (i.e. net offspring fitness; Kokko *et al.*, 2006). However, genetic benefits are context dependent if the performance of different genotypes varies across environments (Roff, 1997). A genotype that is advantageous in one environment may be disadvantageous in another (e.g. Welch, 2003; Hunt & Hosken, 2014). If there are fewer immune threats in eucalypt plantations than in native forests, then natural selection might favour males that invest less in costly immune defences. This could favour females that show no preference for males with a strong immune response. We suggest this argument is biologically implausible, however, as male immune response and sexual trait expression are positively correlated in both habitats (Iglesias-Carrasco *et al.*, 2016) and the relatively recent establishment of eucalypt plantations in the area (< 70 years) seems unlikely to have allowed sufficient time for such a fine-scale adaptive response. Likewise, the distances between natural forests patches and exotic plantations are short enough to be crossed by newts.

### The immediate effect of eucalypt leaf litter on female mate choice

Several studies have shown that toxic substances can impair female mate choice, especially in aquatic

vertebrates (e.g. Park & Propper, 2002; Tomkins *et al.*, 2016). The transmission and reception of sexual signals partly depend on the channel of transmission (Endler, 1992), and male–female communication via pheromones is known to be hindered by the presence of some contaminants. For example, naturally occurring chemicals can bind pheromones together (Mesquita *et al.*, 2003) and block chemoreceptors (Hubbard *et al.*, 2002), thereby affecting the ability to select preferred mates. We showed, however, that mating preferences and the resultant mate choice did *not* change when female palmate newts were tested in eucalypt-infused water. Even when eucalypt leachates were present, females presented with a pair of males still chose the one with the stronger PHA immune response. This result suggests that neither immediate transmission nor reception of palmate newt pheromone signals is inhibited by the presence of eucalypt leachates.

## Conclusions

Sexual selection is affected by environmental factors that influence the condition of choosy individuals. It has been argued that this is especially important in human-modified environments because they often differ dramatically from native habitats in food availability, parasite loads and pollution levels (review: Ditchkoff *et al.*, 2006). We found that leachates reduce the ability of female palmate newts to identify mates with a stronger immune response. Importantly, however, this effect was only detected after females had spent a 21-day period in eucalypt-infused water. This suggests that the effect is driven by changes in female physiology, rather than by the immediate inhibition of transmission or reception of pheromonal signals. Future studies are needed to tease apart the exact effects of eucalypt leachates on female newt physiology if we are to better understand the proximate causes. More generally, human modification of habitat through the planting of eucalypts provides a large-scale ‘natural experiment’ to test how mating preferences evolve when the costs and benefits of mate choice change, and to test the broader effects of long-term consequences of changes in sexual selection at the population level (Power & Holman, 2015).

## Acknowledgments

We thank two reviewers for their help improving the manuscript, Ion Garin-Barrio for field assistance and Aranzadi Society of Sciences for use of their facilities. This work was supported by Basque Government (Basque-Aquitaine Fellow) and the Spanish Ministry of Education and Culture (Predoctoral Grant to M.I-C, FPU12/04148). All capture and handling of newts complied with the existing laws in Spain and were performed under licence from the Álava, and Gipuzkoa

Administrations (numbers 1262, 2222). M.I-C and C.C conducted this work with Spanish accreditation to conduct experiments with animals R.D 53/2013 (ref number 10/096442.9/13).

## References

- Amundsen, T. & Forsgren, E. 2001. Male mate choice selects for female coloration in a fish. *Proc. Natl. Acad. Sci. U.S.A.* **98**: 13155–13160.
- Boughman, J.W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* **411**: 944–948.
- Brodeur, J.C., Suarez, R.P., Natale, G.S., Ronco, A.E. & Elena Zaccagnini, M. 2011. Reduced body condition and enzymatic alterations in frogs inhabiting intensive crop production areas. *Ecotoxicol. Environ. Saf.* **74**: 1370–1380.
- Brooks, R. & Endler, J. 2001. Female guppies agree to differ: phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. *Evolution* **55**: 1644–1655.
- Candolin, U. & Heuschele, J. 2008. Is sexual selection beneficial during adaptation to environmental change? *Trends Ecol. Evol.* **23**: 446–452.
- Candolin, U. & Wong, B.B.M. 2012. *Behavioural Responses To a Changing World: Mechanisms and Consequences*. Oxford University Press, Oxford.
- Candolin, U., Salesto, T. & Evers, M. 2007. Changed environmental conditions weaken sexual selection in sticklebacks. *J. Evol. Biol.* **20**: 233–239.
- Canhoto, C. & Laranjeira, C. 2007. Leachates of *Eucalyptus globulus* in intermittent streams affect water parameters and invertebrates. *Int. Rev. Hydrobiol.* **92**: 173–182.
- Clulow, S., Harris, M. & Mahony, M.J. 2015. Optimization, validation and efficacy of the phytohaemagglutinin inflammation assay for use in ecoimmunological studies of amphibians. *Conserv. Physiol.* **3**: 1–15.
- DeWitt, T.J., Sih, A. & Wilson, D.S. 1998. Cost and limits of phenotypic plasticity. *Trends Ecol. Evol.* **13**: 77–81.
- Ditchkoff, S.S., Saalfeld, S.T. & Gibson, C.J. 2006. Animal behavior in urban ecosystems: modifications due to human-induced stress. *Urban Ecosyst.* **9**: 5–12.
- Endler, J.A. 1992. Signals, signal conditions, and the direction of evolution. *Am. Nat.* **139**: S125–S153.
- Freckleton, R.P. 2001. On the misuse of residuals in ecology: testing regression residual vs the analysis of covariance. *J. Anim. Ecol.* **70**: 708–711.
- Griffiths, R.A. 1985. Diel profile of behaviour in the smooth newt, *Triturus vulgaris* (L.): an analysis of environmental cues and endogenous timing. *Anim. Behav.* **33**: 573–582.
- Head, M.L., Wong, B.B.M. & Brooks, R. 2010. Sexual display and mate choice in an energetically costly environment. *PLoS One* **5**: e15279.
- Heuschele, J., Mannerla, M., Gienapp, P. & Candolin, U. 2009. Environment-dependent use of mate choice cues in sticklebacks. *Behav. Ecol.* **20**: 1223–1227.
- Houck, L.D. 2009. Pheromone communication in amphibians and reptiles. *Annu. Rev. Physiol.* **71**: 161–176.
- Hubbard, P.C., Barata, E.N. & Canario, A.V.M. 2002. Possible disruption of pheromonal communication by humic acid in the goldfish, *Carassius auratus*. *Aquat. Toxicol.* **60**: 169–183.
- Hunt, J., Brooks, R. & Jennions, M.D. 2005. Female mate choice as a condition-dependent life-history trait. *Am. Nat.* **166**: 79–92.

- Hunt, J. & Hosken, D. 2014. *Genotype-by-environment interactions and sexual selection*. John Wiley and Sons, Chichester.
- Iglesias-Carrasco, M., Head, M.L., Jennions, M.D. & Cabido, C. 2016. Condition-dependent trade-offs between sexual traits, body condition and immunity: the effect of novel habitats. *BMC Evol. Biol.* 1–10.
- Jennions, M.D. & Petrie, M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biol. Rev. Camb. Philos. Soc.* **72**: 283–327.
- Kokko, H., Bookmythe, I. & Jennions, M.D. 2015. Mate-sampling costs and sexy sons. *J. Evol. Biol.* **28**: 259–266.
- Kokko, H., Jennions, M.D. & Brooks, R. 2006. Unifying and testing models of sexual selection. *Annu. Rev. Ecol. Evol. Syst.* **37**: 43–66.
- Kwiatkowski, M.A. & Sullivan, B.K. 2002. Geographic variation in sexual selection among populations of an iguanid lizard, *Sauromalus obesus* (=ater). *Evolution* **56**: 2039–2051.
- Larrañaga, A., Basaguren, A., Elozegi, A. & Pozo, J. 2009a. Impacts of *Eucalyptus globulus* plantations on Atlantic streams: changes in invertebrate density and shredder traits. *Fundam. Appl. Limnol.* **175**: 151–160.
- Larrañaga, A., Basaguren, A. & Pozo, J. 2009b. Impacts of *Eucalyptus globulus* plantations on physiology and population densities of invertebrates inhabiting Iberian Atlantic streams. *Int. Rev. Hydrobiol.* **94**: 497–511.
- Lochmiller, R.L., Vestey, M.R. & Boren, J.C. 1993. Relationship between protein nutritional status and immunocompetence in northern bobwhite chicks. *Auk* **110**: 503–510.
- Maan, M.E., Hofker, K.D., van Alphen, J.J.M. & Seehausen, O. 2006. Sensory drive in cichlid speciation. *Am. Nat.* **167**: 947–954.
- Maerz, J.C., Brown, C.J., Chapin, C.T. & Blossey, B. 2005. Can secondary compounds of an invasive plant affect larval amphibians? *Funct. Ecol.* **19**: 970–975.
- Martin, L.J. & Blossey, B. 2013. Intraspecific variation overrides origin effects in impacts of litter-derived secondary compounds on larval amphibians. *Oecologia* **173**: 449–459.
- Mesquita, R.M.R.S., Canário, A.V.M. & Melo, E. 2003. Partition of fish pheromones between water and aggregates of humic acids. Consequences for sexual signaling. *Environ. Sci. Technol.* **37**: 742–746.
- Milinski, M., Griffiths, S., Wegner, K.M., Reusch, T.B.H., Haas-Assenbaum, A. & Boehm, T. 2005. Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. *Proc. Natl. Acad. Sci. U.S.A.* **102**: 4414–4418.
- Møller, A., Christe, P. & Lux, E. 1999. Parasitism, host immune function, and sexual selection. *Q. Rev. Biol.* **74**: 3–20.
- Montori, A. & Herrero, P. 2004. Caudata. In: *Amphibia. Lis-samphibia. Fauna Ibérica 24* (M. García-París et al., eds), pp. 43–275. Museo Nacional de Ciencias Naturales (CSIC), Madrid.
- Morrongiello, J.R., Bond, N.R., Crook, D.A. & Wong, B.B.M. 2011. *Eucalyptus* leachate inhibits reproduction in a freshwater fish. *Freshw. Biol.* **56**: 1736–1745.
- Morrongiello, J.R., Bond, N.R., Crook, D.A. & Wong, B.B.M. 2013. Intraspecific variation in the growth and survival of juvenile fish exposed to *Eucalyptus* leachate. *Ecol. Evol.* **3**: 3855–3867.
- Murillo-Rincón, P.A., Laurila, A. & Orizaola, G. 2017. Compensating for delayed hatching reduces offspring immune response and increases life-history costs. *Oikos* **126**: 565–571.
- Navarro, C., Marzal, A., de Lope, F. & Moller, A.P. 2003. Dynamics of an immune response in house sparrows *Passer domesticus* in relation to time of day, body condition and blood parasite infection. *Oikos* **101**: 291–298.
- Park, D. & Propper, C.R. 2002. Endosulfan affects pheromonal detection and glands in the male red-spotted newt, *Notophthalmus viridescens*. *Bull. Environ. Contam. Toxicol.* **69**: 609–616.
- Park, D., Hempleman, S.C. & Propper, C.R. 2001. Endosulfan exposure disrupts pheromonal systems in the red-spotted newt: a mechanism for subtle effects of environmental chemicals. *Environ. Health Perspect.* **109**: 669–673.
- Power, D.J. & Holman, L. 2015. Assessing the alignment of sexual and natural selection using radiomutagenized seed beetles. *J. Evol. Biol.* **25**: 1039–1048.
- Rafferty, N.E. & Boughman, J.W. 2006. Olfactory mate recognition in a sympatric species pair of three-spined sticklebacks. *Behav. Ecol.* **17**: 965–970.
- Roff, D. 1997. *Evolutionary quantitative genetics*. Chapman and Hall, New York.
- Schmid-Hempel, P. 2003. Variation in immune defence as a question of evolutionary ecology. *Proc. Biol. Sci.* **270**: 357–366.
- Sparling, D., Linder, G., Bishop, C. & Krest, S. 2010. *Ecotoxicology of Amphibians and Reptiles*. CRC Press, New York, NY, USA.
- Steinberg, C.E.W., Kamara, S., Prokhotskaya, V.Y., Manusadzianas, L., Karasyova, T.A., Timofeyev, M.A. et al. 2006. Dissolved humic substances – ecological driving forces from the individual to the ecosystem level? *Freshw. Biol.* **51**: 1189–1210.
- Temmink, J.H.M., Field, J.A., van Haastrecht, J.C. & Merklbach, R.C.M. 1989. Acute and sub-acute toxicity of bark tannins in carp (*Cyprinus carpio* L.). *Water Res.* **23**: 341–344.
- Tomkins, P., Saaristo, M., Allinson, M. & Wong, B.B.M. 2016. Exposure to an agricultural contaminant, 17 $\beta$ -trenbolone, impairs female mate choice in a freshwater fish. *Aquat. Toxicol.* **170**: 365–370.
- Watling, J.I., Hickman, C.R., Lee, E., Wang, K. & Orrock, J.L. 2011. Extracts of the invasive shrub *Lonicera maackii* increase mortality and alter behavior of amphibian larvae. *Oecologia* **165**: 153–159.
- Welch, A.M. 2003. Genetic benefits of a female mating preference in gray tree frogs are context-dependent. *Evolution* **57**: 883–893.
- Wong, B.B.M. & Jennions, M.D. 2003. Costs influence male mate choice in a freshwater fish. *Proc. Biol. Sci.* **270**(Suppl): S36–S38.

## Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article: **Figure S1** Histograms of the residuals of the model and raw data from experiment 1.

**Figure S2** Histograms of the residuals of the model and raw data from experiment 2.

Data deposited at Dryad: doi:10.5061/dryad.k669c

Received 20 December 2016; revised 26 March 2017; accepted 7 April 2017