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Long-term captivity is associated with changes to sensory organ morphology in a critically endangered insect

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Abstract

1. Captive breeding programs are key to many threatened species reintroduction strategies but could potentially be associated with adaptations to captivity that are maladaptive in their natural habitat. Despite the importance of sensory ecology to biological fitness, few studies explore sensory system adaptations to captivity. Captive environments are devoid of predators and provide ready access to food sources and potential mates, thus reducing the need for individuals to use signals and cues to identify and locate resources or detect potential threats. With reduced complexity of the signalling environment, relaxation of selective pressures may favour reduced investment in sensory organs in captivity.
2. We test this prediction in an iconic critically endangered invertebrate, the Lord Howe Island stick insect *Dryococelus australis*, which was extirpated from the island in the 1920s/30s and rediscovered on a nearby volcanic stack, Ball's Pyramid, in 2001.
3. Using historical specimens from these populations and specimens from the 8–10th and 14th generations of a long-term conservation captive breeding program, we examine differences in behaviourally relevant morphological traits of the compound eyes (visual organs) and antennae (olfactory organs).
4. We find that captivity is associated with smaller compound eye size, smaller eye ommatidia and reduced density of antennal odour receptors. These morphological changes are indicative of reduced sensitivity to visual and olfactory signals and cues, and therefore are likely to have fitness implications when reintroducing a captive population into the wild.
5. Synthesis and applications: We observe differences in sensory organ morphology between wild and captive bred populations of the critically endangered Lord Howe Island stick insect. Our results emphasise the importance of incorporating evolutionary biology and sensory ecology into conservation program design: to minimise the potential for captive breeding environments to compromise sensory systems that support appropriate behaviours upon reintroduction of populations into a natural habitat.

Keywords: antenna, captive breeding, compound eye, conservation, *Dryococelus australis*, Lord Howe Island stick insect, sensory ecology

Introduction

We are amidst the Earth's sixth mass extinction event (Barnosky et al., 2011), with an unprecedented number of species being driven to extinction via rapid environmental change resulting from anthropogenic activities (Dirzo et al., 2014). The increasing number of threatened species (IUCN, 2021) has encouraged a multitude of conservation strategies, one of the more important of which is captive breeding programs for species reintroductions (Seddon et al., 2007). Such breeding programs, established with individuals from wild populations of threatened species, provide insurance against extinction (Jakob-Hoff et al., 2015) and are commonly used to reinforce existing wild populations or to provide a founding population to re-introduce the species once threats are removed (IUCN/SSC, 2013; Jakob-Hoff et al., 2015). Captive breeding programs provide an environment typically free from extinction drivers and provide the opportunity for program managers to regulate reproduction to retain genetic diversity, and thus increase the likelihood of the successful establishment of new wild populations (Frankham, 1995; Weeks et al., 2015). While most often used for vertebrate species, captive breeding programs are increasingly used as conservation strategies for invertebrates (Dojnov et al., 2012; Holwell & Andrew, 2015; Honan, 2007; Leather et al., 2008; Pearce-Kelly et al., 1998; Stringer & Chappell, 2008).

Captive breeding programs typically create benign living environments that can result in selection for survival in environments that have little resemblance to natural habitats (Frankham et al., 2010; Lacy, 1987; Williams & Hoffman, 2009). This can have significant fitness consequences for individuals subsequently released into the wild, with adaptations to captivity in these contexts typically being non-adaptive in the natural environment (Lewis & Thomas, 2001; Sutherland, 1998) and often resulting from the relaxation of natural selection pressures in captivity. Invertebrates are vulnerable to such effects (Dojnov et al., 2012; Frankham & Loebel, 1992; Lewis & Thomas, 2001; Woodworth et al., 2002), especially due to their comparatively short generation times (Lewis & Thomas, 2001). Studies of adaptation to captivity focus mostly on anti-predator responses (Kraaijeveld-Smit et al., 2006) and reproductive traits (Frankham & Loebel, 1992; Heath et al., 2003; Joron & Brakefield, 2003; Lewis & Thomas, 2001; Woodworth et al., 2002), with other morphological traits usually analysed only in the context of life history trade-offs with reproductive investment (Lewis & Thomas, 2001). Despite the importance of considering sensory ecology when designing and implementing conservation strategies (Lim et al., 2008), sensory system adaptations to captivity have not been investigated in a conservation context.

Animals depend on their ability to detect information from their environment, including the location of appropriate food sources, potential mates or approaching predators. Insects have diverse and complex sensory organs to achieve this (Elgar et al., 2018): the ommatidia (facets) of the compound eye are the primary sensory receptors for detecting visual cues, and the sensilla on the antennae are used to detect odours, movement and tactile information. Elaborate sensory systems require considerable energetic resources to develop and maintain, due largely to the associated neural circuitry (Niven & Laughlin, 2008), and sensory organ morphology is optimised to detect salient signals and cues from the background noise in the signalling environment (Elgar et al., 2018; Endler, 1992). For example, insects living in environments characterised by low ambient light levels have larger compound eye ommatidia to enhance sensitivity to light (Freelance et al., 2021) while halictid bees that evolved from a social to solitary lifestyle, and thus no longer need to frequently detect diverse social odours, have a lower density of antennal sensilla (Wittwer et al., 2017). Captive breeding environments, typically characterised by ready access to suitable food sources, proximity of potential mates and an absence of predators, effectively simplify the sensory environment and thus may relax natural selection pressures on sensory morphology that would be present in the wild. Accordingly, selection should favour changes to the morphology of sensory organs such that they are optimised (sufficiently but not unnecessarily sensitive) and/or adapted to the signal detection requirements of this new, sensory depauperate, environment.

To test this prediction, we explored differences in sensory organ morphology between wild and captive bred populations of a critically endangered insect for which a captive conservation breeding program has been ongoing since 2003. The iconic Lord Howe Island stick insect (LHISI), *Dryococelus australis* (Phasmatodea: Phasmatidae), is a large, black, flightless phasmid that was historically endemic to Lord Howe Island off the coast of New South Wales, Australia (31°33’15” S, 159°05’06” E) (Lea, 1916). Rats were accidentally introduced to the island in a 1918 shipwreck, leading to the supposed extinction of the insect in the 1920s (Priddel et al., 2003). However, a small population of the LHISI was re-discovered some 80 years later on a nearby volcanic stack, Ball’s Pyramid (31°45′15″ S, 159°15′06″ E) (Priddel et al., 2003). Recent genetic studies confirmed that the stick insects on Ball’s Pyramid are the LHISI (Mikheyev et al., 2017). In 2003, two adult breeding pairs were removed from Ball’s Pyramid to start a captive conservation breeding program at Zoos Victoria’s Melbourne Zoo (Parkville, Victoria, Australia) and at Insektus (Sydney, New South Wales, Australia) (Carlile et al., 2009; Honan, 2007). The Melbourne Zoo population, currently maintained free-ranging in glasshouses, reached its 14th captive bred generation in 2018. This captive population is intended to be the source of LHISI for reintroduction to Lord Howe Island (Bower et al., 2018) following a rodent eradication program in 2019 (Lord Howe Island Board, 2020).

The Lord Howe Island, Ball’s Pyramid and captive environments differ in the complexity of the sensory environment. Firstly, Lord Howe Island has diverse vegetation with which the LHISI historically interacted, including both food and non-food plants (Honan, 2008; McGrath et al., 2017), while the LHISI on Ball's Pyramid is known to associate only with the Lord Howe Island Melaleuca *Melaleuca howeana*. This plant is also one of only a few host (food and shelter) plant species provided to the Melbourne Zoo captive population (Honan, 2008; McGrath et al., 2017), meaning that both the Ball's Pyramid and the captive populations rarely use odours to differentiate among food and non-food plants. Secondly, the captive breeding environment is devoid of potential predators in contrast with Lord Howe Island (spiders, birds, and small mammals) and Ball's Pyramid (seabirds), and so captive bred individuals are not disadvantaged if they lose sensitivity to predator-related cues. Thirdly, while there is evidence of gregarious living from both the wild (Lea, 1916) and captive (Honan, 2008) populations, the maximum possible distance between two individuals in the captive breeding environment is significantly reduced, thereby reducing reliance on location-revealing sex pheromones to locate a mate.

The complexity of the sensory environment is evidently greater on Lord Howe Island than on Ball's Pyramid and is least for populations bred in captivity. Accordingly, we predicted that (i) individuals from the Lord Howe Island wild population (pre-extirpation) will have morphology indicative of greater sensitivity of the compound eyes and antennae compared to individuals from the Ball's Pyramid wild population and (ii) captive breeding will be associated with morphology indicating reduced sensitivity of the compound eyes and antennae compared to both wild populations.

Materials and Methods

Study populations

We accessed, from the Australian Museum entomology collection (Sydney, Australia), ethanol-preserved historical specimens from both the Lord Howe Island (LHI) and Ball's Pyramid (BP) wild populations. Seven specimens (n = 4 females; 3 males) had been collected from Lord Howe Island in the late 1800s pre-extirpation, and we examined the only two available specimens (n = 1 female; 1 male) of the four individuals collected from Ball's Pyramid in 2003 to establish the captive populations (Honan, 2007). The latter pair is believed to be the individuals provided to the Insektus organisation: we were unable to locate the breeding pair which founded the Melbourne Zoo captive population in any museum or zoo collections.

We examined the effects of long-term captive breeding on sensory morphology over generations by accessing representative specimens from two generations of the Melbourne Zoo captive population. These specimens had been preserved by freezing from 2011–2013, providing us with individuals (n = 10 females; 5 males) from generations 8–10 of captive breeding (MZ generations 8–10) since the establishment of the population with wild stock from Ball's Pyramid. In late 2018 when this study was initiated, the invertebrate keepers collected and froze all naturally deceased individuals until the end of that year, providing us with 6 females and 9 males from the 14th captive bred generation (MZ generation 14). Only adult stick insects were included in the study. Our study did not require animal research ethics approval.

Data collection

Eye ommatidia is positively associated with sensitivity to light (Jander & Jander, 2002; Land, 1997; Warrant, 2017) and eye size can indicate investment in photic sensitivity versus visual acuity, as a larger compound eye with smaller but more numerous ommatidia theoretically has greater visual acuity (Jander & Jander, 2002). Antennal sensilla density is a behaviourally relevant indicator of sensitivity to olfactory and tactile cues (Elgar et al., 2018; Gill et al., 2013; Spaethe et al., 2007). Therefore, we used these three metrics of sensory capacity to compare across the study populations. Only undamaged eyes or antennae were analysed.

The compound eyes were imaged using a Leica MZ16 A stereomicroscope with Leica DFC500 camera (Leica Microsystems) at the Australian Museum (Sydney, Australia) or a Leica M205 stereomicroscope with Leica DFC500 camera at the BioSciences Microscopy Unit (The University of Melbourne, Australia). Using the images, we determined for each specimen the surface area of the compound eye (calculated as half of the surface area of a spheroid with semi-axes equivalent to the length and depth of the compound eye; mm^2) and the average diameter of the ommatidia of the compound eye (diameter of three ommatidium averaged; µm). As differences in ommatidia diameter between regions of the compound eye are not uncommon (Perl & Niven, 2016), for consistency we measured ommatidia from the dorso-medial (skyward-facing) region of the compound eyes.

To image the antennae, the left antenna was removed from each specimen and affixed on black matte cardboard on a scanning electron microscope stub using double-sided carbon sticky dots. If the left antenna was not intact for a specimen, the intact right antenna was used to maximise sample size. Mounted antennae were made conductive by gold coating using a Dynavac Xenosput gold coater and subsequently imaged using a FEI/Philips XL30 FEG scanning electron microscope (10kV acceleration voltage, spot size 3.0) at the BioSciences Microscopy Unit. From the electron micrographs, we determined for each specimen the average density of each type of antennal sensilla (number of sensilla per mm^2 of antenna) on the apical (1st) and 8th-most apical antennal segments (antennomers). As the antennal sensilla of the LHISI have not been previously examined, we first had to identify and describe the sensilla present before we could calculate sensilla density to compare across populations. Antennal sensilla were classified into four categories: olfactory/chemoreceptive sensilla detect airborne odours and chemicals in solution (Slifer, 1966), hair-like mechanoreceptive sensilla (tactile hairs) are involved in the detection and localisation of objects in the near-range environment during antennation (Dürr & Krause, 2013), campaniform sensilla detect stretch forces due to mechanical deformation of the cuticle due to external forces or movement of the antenna initiated by the insect (Chapman, 1998; Zill et al., 2011), and hygro- and thermo-receptive sensilla (sensory pores) detect changes in humidity and temperature. Antenna length was not measured as meaningful comparison of this metric was precluded by the antennae having an inconsistent number of antennomers and by the inconsistent length of antennomers.

Using a Canon 6D DSLR with Canon EF-L 100mm f2.8 macro lens (Canon, Tokyo, Japan), we took digital images of the femurs of each individual, with a ruler included as a scale, as a measure of body size. All image analyses were performed using the software package FIJI (Schindelin et al., 2012).

Statistical analysis

One sensory trait, the density of campaniform sensilla, required natural log transformation to normalise the distribution for an ANOVA. For each sensory trait, we fitted a linear model including population (LHI, BP, MZ generations 8–10, MZ generation 14), sex (female, male) and body size as fixed effects with variance partitioned using ordinary least squares. In the event of a significant ANOVA (type III) *F* test for population, four planned pairwise comparisons were performed with any significant differences reported: LHI against BP to explore difference between the two wild populations; BP against MZ generations 8–10 to explore differences between the source population and the closest available generations of the derived captive population; MZ generations 8–10 against MZ generation 14 to explore changes across generations in captivity; MZ generation 14 against LHI as MZ generation 14 represents the most recent studied generation of the captive population which may be introduced onto Lord Howe Island. Statistical analysis was performed using the CAR (version 3.0-11) (Fox & Weisberg, 2019), EFFECTSIZE (version 0.4.5) (Ben-Shachar et al., 2020) and MULTCOMP (version 1.4-17) (Hothorn et al., 2008) packages in R version 4.1.0 for Windows (R Core Team, 2021).

Results

Description of antennal sensilla morphology

We identified seven types of antennal sensilla (Fig. 1): three types of chemoreceptive sensilla (sensilla basiconica, thick-walled chemoreceptors (TWC), sensilla trichotomous), two types of tactile hairs (sensilla trichodea, sensilla chaetica), one type of plate-like mechanoreceptive sensilla (sensilla campaniforma), and one type of pore-like thermo- and hygro-receptors (sensilla coeloconica). Because trichotomous sensilla were only identified on some specimens from the MZ captive population and their function is uncertain, they were excluded from the sensilla density analysis. Descriptions of the morphological characteristics of each sensillum type identified are in Table S1 in Supporting Information.

Population differences in sensory organ morphology

As predicted, the surface area of the compound eye was explained by population ($F_{3,31} = 3.354$, $p = 0.031$, $\eta^2_p = 0.25$ (0.00, 0.45) (partial eta squared (95% confidence intervals))) (Fig. 2A): planned pairwise comparisons reveal the LHI population had significantly larger eyes than MZ generation 14 population ($t = 2.235$, $p = 0.033$). Eye size did not differ significantly by sex ($F_{1,31} = 4.132$, $p = 0.051$, $\eta^2_p = 0.12$ (0.00, 0.35)) or with femur length as a measure of body size ($F_{1,31} = 1.05$, $p = 0.314$, $\eta^2_p = 0.03$ (0.00, 0.22)).

Consistent with our prediction, the diameter of the ommatidia of the compound eye also varied significantly by population ($F_{3,32} = 4.491$, $p = 0.0097$, $\eta^2_p = 0.30$ (0.03, 0.49); Fig. 2B): planned pairwise comparisons reveal the LHI population had significantly larger ommatidia than the wild BP population ($t = 2.931$, $p = 0.006$) and the captive MZ generation 14 ($t = 2.056$, $p = 0.048$). Females had significantly larger ommatidia than males (females: 68.31 ± 1.316 µm (mean ± SE), males: 58.38 ± 1.505 µm, $F_{1,32} = 9.470$, $p = 0.004$, $\eta^2_p = 0.23$ (0.03, 0.45)), but ommatidia diameter was not explained by femur length ($F_{1,32} = 0.381$, $p = 0.542$).

The density of chemoreceptive sensilla on the apical antennomer differed significantly by population ($F_{3,30} = 2.984$, $p = 0.047$, $\eta^2_p = 0.23$ (0.00, 0.44); Fig. 2C), with planned pairwise comparisons revealing a significantly higher density in the LHI population compared to the captive MZ generation 14 ($t = 2.925$, $p = 0.007$). It should be noted that the LHI sample for this trait includes an outlier leveraging the result; this value may indicate the existence of even greater variation in the extirpated LHI population that would be apparent in a larger sample. Separate analyses of each type of chemoreceptor – TWCs and sensilla basiconica – were conducted to determine which type drive the pattern. The density of sensilla basiconica on the apical antennomer did not differ between populations ($F_{3,30} = 0.574$, $p = 0.637$) but the density of TWCs did ($F_{3,30} = 2.972$, $p = 0.048$, $\eta^2_p = 0.23$ (0.00, 0.44); Fig. 2D): the LHI population had a higher TWC density than MZ generation 14 ($t = 2.844$, $p = 0.008$). The density of chemoreceptive sensilla on the apical antennomer was also related to sex (females: 523.9 ± 32.93 sensilla/mm^2, males: 585.5 ± 38.02, $F_{1,30} = 6.344$, $p = 0.017$, $\eta^2_p = 0.17$ (0.00, 0.41)), but sex did not explain the density of sensilla basiconica ($F_{1,30} = 3.435$, $p = 0.074$, $\eta^2_p = 0.10$ (0.00, 0.33)) or TWCs ($F_{1,30} = 4.022$, $p = 0.054$, $\eta^2_p = 0.12$ (0.00, 0.35)) individually. The density of chemoreceptive sensilla, and of TWCs and sensilla basiconica when analysed separately, was negatively associated with body size: smaller individuals had higher densities (chemoreceptors: $\beta = -28.59$, $F_{1,30} = 13.26$, $p = 0.001$, $\eta^2_p = 0.31$ (0.07, 0.53); sensilla basiconica: $\beta = -10.30$, $F_{1,30} = 9.535$, $p = 0.004$, $\eta^2_p = 0.24$ (0.03, 0.47); TWCs: $\beta = -18.28$, $F_{1,30} = 7.278$, $p = 0.011$, $\eta^2_p = 0.20$ (0.01, 0.43)).

The variation in the densities of no other antennal sensilla, on either the apical or 8th antennomer, was explained by population, sex or body size (Table 1). The 8th antennomer density of sensory pores was positively associated with femur length ($\beta = 24.40$, $F_{1,28} = 4.273$, $p = 0.048$, $\eta^2_p = 0.13$ (0.00, 0.37)).

Discussion

Our results support the prediction that the simplification of the sensory environment, reflective in the captive and, to a lesser extent, BP populations, selects for smaller eyes and/or ommatidia and for antennae with a lower density of chemoreceptors. Investment in sensory organs is expected to reflect a balance between the energetic costs of sensory organs (Niven & Laughlin, 2008) and their capacity to detect salient cues and signals against the background noise of the sensory environment (Endler, 1992). Investing in less elaborate and costly sensory organs in comparatively less complex sensory environments allows individuals to redirect energetic resources to other fitness related traits. These changes in sensory organ morphology associated with the simplified wild (BP) or captive (MZ) sensory environment could be the result of evolutionary change or phenotypic plasticity. Indeed, plasticity is often proposed to precede, and possibly facilitate, evolutionary adaptation (Levis & Pfennig, 2016) and is known to drive changes in insect sensory organ morphology (Bernays & Chapman, 1998; Chapman, 2002). Elucidating whether the patterns we observe are fixed or plastic would require experimental manipulations such as changing the complexity of the captive breeding environment and assessing sensory organ morphology over subsequent generations; the limited captive population size and husbandry requirements of this highly threatened species make such an experiment challenging. Regardless of whether the differences we observe reflect evolutionary change or plasticity (Hendry et al., 2008; Hendry et al., 2017), our data are consistent with the notion that the complexity of the sensory environment influences sensory organ morphology and our findings regarding eye size in particular support growing evidence of adaptations to breeding in captivity (Dojnov et al., 2012; Frankham & Loebel, 1992; Heath et al., 2003; Joron & Brakefield, 2003; Kraaijeveld-Smit et al., 2006; Lewis & Thomas, 2001; Woodworth et al., 2002).

The reduced compound eye surface area in the captive population of the LHISI may be due to an absence of predators in captivity: a relatively large compound eye with many ommatidia theoretically confers greater visual acuity, and larger ommatidia provide greater photon capture ability (Jander & Jander, 2002). Visual acuity and visual sensitivity are likely of greater benefit in populations that need to detect approaching predators, such as birds. In contrast, individuals reared in captivity experience no selection pressure to detect predators. As each ommatidium is associated with energetically expensive neural architecture required to detect photons and convey this information to the brain to form a visual map (Agi et al., 2014; Niven & Laughlin, 2008), a simultaneous decrease in overall eye size reduces the energetic cost of the eye.

While leveraged by a statistical outlier in the extirpated LHI population, the between-population differences in antennal sensilla density are consistent with our prediction. This was driven by the TWC type of chemoreceptor which is associated with detecting airborne odours and compounds in solution (Slifer, 1966) and is likely the predominant sensilla for detection of plant volatiles and pheromones. *D. australis* feeds on a range of food plants found on LHI but clearly prefers a few species (McGrath et al., 2017), whereas *M. howeana* may be the only food plant on BP and is the most frequently provided in captivity (Honan, 2008; McGrath et al., 2017). Perhaps individuals on LHI relied on plant odours to identify and locate their preferred food plants, while those on BP and in captivity have little or no requirement to exercise a choice. Dietary composition can also influence the distribution of chemoreceptors on the antennae of invertebrates (Bernays & Chapman, 1998; Chapman, 2002). Finally, the maximum possible distance between females and males in the BP and captive environments is substantially less than that on LHI, perhaps eliminating the need for males (with a higher density of chemoreceptors than females) to rely heavily on location-revealing female sex pheromones.

While the patterns of sensory organ investment across the populations are consistent with our prediction and with differences in the sensory environment, other variables may also be responsible. Firstly, museum specimens often derive from opportunistic or selective collecting which may be biased towards larger specimens (Pyke & Ehrlich, 2010). This could explain why specimens from LHI possess larger eyes and higher densities of antennal sensilla compared to the captive population specimens that were more representative of the population. However, the absence of positive body size allometry with eye or antennal metrics suggests that such bias is unlikely to explain our results. Secondly, our LHI and especially BP sample sizes are very small and may not be an accurate representation of the entire population. Small sample sizes for these populations, while generally unavoidable with critically endangered species, may also have impacted our ability to detect statistically all population differences. Thirdly, the captive population, known to be derived from a maximum of only four individuals, is likely to have been influenced by a founder effect and will have very limited genetic variation; this may also be true of the BP population, which has an unknown number of founders (indeed, the separation time between the BP and extirpated LHI populations is unknown (Mikheyev et al., 2017) and there is uncertainty whether differences between the BP and LHI populations result from historical founder effects). Consequently, through founder effects there may have been insufficient genetic diversity for adaptation (as opposed to plasticity) to explain the differences we observe between the wild and captive populations. This could account for the absence of differences between the 8–10th and 14th generations of the MZ captive population in any of the traits we examined, although it is also possible that the sensory morphology of the 8–10th generations had already become optimised to the captive environment and thus remained unchanged in subsequent generations. Inbreeding depression, resulting from low genetic variation and multiple generations in captivity, could also contribute to sub-optimal expression of morphological traits. Despite these caveats, our data provide novel practical insights from a real-world conservation captive breeding program of a critically endangered species, and thus relate to an actual situation that must be managed rather than being derived from a laboratory experiment with a model organism.

Differences in sensory organ morphology in captive compared with natural populations have implications for species reintroduction programs, since reintroduced individuals adapted to captivity may well be less equipped to respond to their new, natural, environment (Lewis & Thomas, 2001; Sutherland, 1998). However, captive populations, especially if added to with further founders over the course of the breeding program, may retain sufficient genetic diversity to respond to selection pressures when reintroduced into the natural environment. Moreover, if the differences reflect plasticity rather than solely evolutionary change (Hendry et al., 2008), the recovery of visual and olfactory sensitivity following reintroduction could be rapid. Changes to captive population husbandry, such as increasing the complexity of the sensory environment by presenting a variety of both food and non-food plants in captivity, may facilitate changes in the direction of greater sensitivity before wild reintroduction begins. Additionally, a dietary composition similar to that of the natural habitat may further favour more appropriate antennal morphology due to the direct influence of diet on antennal chemoreceptor density (Bernays & Chapman, 1998; Chapman, 2002). Presenting a variety of plants similar to those in the natural habitat may also facilitate greater expression of natural exploratory behaviours in captivity, which promotes general welfare (Freelance, 2019). In the case of the LHISI, introducing a variety of food and non-food plants is unlikely to compromise the fitness of the captive population, as captive individuals can thrive on a variety of food plants native to LHI (McGrath et al., 2017).

In conclusion, our findings from an ongoing conservation captive breeding program are consistent with the prediction that long-term captive breeding of invertebrates may be associated with adaptation of sensory organs to the captive environment. With the increasing use of captive breeding programs as part of threatened species insurance and recovery strategies, these findings provide a cautionary tale: that simplified environments may compromise sensory systems that support efficient expression of appropriate behaviours in a natural habitat. These results highlight the need to consider invertebrate models in evaluating captive breeding and reintroduction programs, emphasise the importance of employing evolutionary biology when undertaking such programs for species reintroductions, and echo long-neglected calls to consider sensory ecology when designing conservation programs (Lim et al., 2008).

Authors' contributions: CF conceived of the study; CF, BW, ME and MM designed the study; CF and MM sourced the specimens; CF performed the data collection and analysis and drafted the manuscript; all authors contributed to preparation of the final manuscript. All authors are scientists from the country in which the research was conducted and to which the study species is endemic.

Conflict of Interest: The authors have no conflicts to declare.

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Data availability statement: Data available via figshare https://doi.org/10.26188/15155607 (Freelance, Magrath, Elgar, & Wong, 2021).

References

Agi, E., Langen, M., Altschuler, S. J., Wu, L. F., Zimmermann, T., & Hiesinger, P. R. (2014). The evolution and development of neural superposition. *Journal of Neurogenetics, 28(3–4),* 216–232. https://doi.org/10.3109/01677063.2014.922557

Barnosky, A. D., Matzke, N., Tomiya, S., Wogan, G. O. U., Swartz, B., Quental, T. B., Marshall, C., McGuire, J. L., Lindsey, E. L., Maguire, K. C., Mersey, B., & Ferrer, E. A. (2011). Has the Earth's sixth mass extinction already arrived? *Nature, 471(7336)*, 51–57. https://doi.org/10.1038/nature09678

Ben-Shachar, M., Lüdecke, D., & Makowski, D. (2020). effectsize: Estimation of effect size indices and standardized parameters. *Journal of Open Source Software, 5(56)*, 2815. https://doi.org/10.21105/joss.02815

Bernays, E. A., & Chapman, R. F. (1998). Phenotypic plasticity in numbers of antennal chemoreceptors in a grasshopper: effects of food. *Journal of Comparative Physiology A, 183*, 69–76.

Bower, H., Carlile, N., Cleave, R., Haselden, C., Hiscox, D., & O'Neill, L. (2018). The path to recovery for the 'extinct' Lord Howe Island phasmid. In S. Garnett, P. Latch, D. Lindenmeyer, & J. Woinarski (Eds.), *Recovering Australian threatened species: a book of hope* (pp. 198). CSIRO Publishing.

Carlile, N., Priddel, D., & Honan, P. (2009). The recovery programme for the Lord Howe Island Phasmid (*Dryococelus australis*) following its rediscovery. *Ecological Management & Restoration, 10,* S124–S128. https://doi.org/10.1111/j.1442-8903.2009.00450.x

Chapman, R. F. (1998). The Insects: Structure and function (4th ed.). Cambridge University Press.

Chapman, R. F. (2002). Development of phenotypic differences in sensillum populations on the antennae of a grasshopper, *Schistocerca americana*. *Journal of Morphology, 254,* 186–194.

Dirzo, R., Young, H. S., Galetti, M., Ceballos, G., Isaac, N. J. B., & Collen, B. (2014). Defaunation in the Anthropocene. *Science, 345(6195),* 401–406. https://doi.org/10.1126/science.1251817

Dojnov, B., Vujčić, Z., Božić, N., Margetić, A., Vujčić, M., Nenadović, V., & Ivanović, J. (2012). Adaptations to captive breeding of the longhorn beetle *Morimus funereus* (Coleoptera:

Cerambycidae); application on amylase study. *Journal of Insect Conservation, 16(2),* 239–247. https://doi.org/10.1007/s10841-011-9411-x

Dürr, V., & Krause, A. F. (2013). Tactile sensing in insects. In D. Jaeger & R. Jung (Eds.), *Encyclopedia of Computational Neuroscience* (pp. 1–5). Springer New York.

Elgar, M. A., Zhang, D., Wang, Q., Wittwer, B., Thi Pham, H., Johnson, T. L., Freelance, C. B., & Coquilleau, M. (2018). Insect antennal morphology: the evolution of diverse solutions to odorant perception. *The Yale Journal of Biology and Medicine, 91(4),* 457–469.

Endler, J. A. (1992). Signals, signal conditions, and the direction of evolution. *The American Naturalist, 139,* S125-S153. https://doi.org/10.1086/285308

Fox, J., & Weisberg, S. (2019). An {R} Companion to Applied Regression (3rd ed.). Sage.

Frankham, R. (1995). Genetic management of captive populations for reintroduction. In M. Serena (Ed.), *Reintroduction biology of Australian and New Zealand fauna* (pp. 31–34). Surrey Beatty and Sons.

Frankham, R., Ballou, J. D., & Briscoe, D. A. (2010). Introduction to Conservation Genetics (2nd ed.). Cambridge University Press.

Frankham, R., & Loebel, D. A. (1992). Modeling problems in conservation genetics using captive *Drosophila* populations: Rapid genetic adaptation to captivity. *Zoo Biology, 11(5),* 333–342. https://doi.org/10.1002/zoo.1430110505

Freelance, C. B. (2019). To regulate or not to regulate? The future of animal ethics in experimental research with insects. *Science and Engineering Ethics, 25(5),* 1339–1355. https://doi.org/10.1007/s11948-018-0066-9

Freelance, C. B., Magrath, M. J. L., Elgar, M. A., & Wong, B. B. M. (2021). Data from: Long-term captivity is associated with changes to sensory organ morphology in a critically endangered insect. *fighare*, https://doi.org/10.26188/15155607

Freelance, C. B., Tierney, S. M., Rodriguez, J, Stuart-Fox, D. M., Wong, B. B. M., & Elgar, M. A. (2021). The eyes have it: dim-light activity is associated with the morphology of eyes but not antennae across insect orders. *Biological Journal of the Linnean Society, 134,* 303–315. https://doi.org/10.1093/biolinnean/blab088

Gill, K. P., van Wilgenburg, E., Macmillan, D. L., & Elgar, M. A. (2013). Density of antennal sensilla influences efficacy of communication in a social insect. *The American Naturalist, 182,* 834–840. https://doi.org/10.1086/673712

Heath, D. D., Heath, J. W., Bryden, C. A., Johnson, R. M., & Fox, C. W. (2003). Rapid evolution of egg size in captive salmon. *Science, 299(5613),* 1738–1740. https://doi.org/10.1126/science.1079707.

Hendry, A. P., Farrugia, T. J., & Kinnison, M. T. (2008). Human influences on rates of phenotypic change in wild animal populations. *Molecular Ecology, 17*, 20–29. https://doi.org/10.1111/j.1365-294X.2007.03428.x

Hendry, A. P., Gotanda, K. M., & Svensson, E. I. (2017). Human influences on evolution, and the ecological and societal consequences. *Philosophical Transactions of the Royal Society B: Biological Sciences, 372(1712)*, 20160028. https://doi.org/10.1098/rstb.2016.0028

Holwell, G. I., & Andrew, N. R. (2015). Protecting the small majority: insect conservation in Australia and New Zealand. In A. Stow, N. Maclean, & G. I. Holwell (Eds.), *Austral Ark: the state of wildlife in Australia and New Zealand* (pp. 278–297). Cambridge University Press.

Honan, P. (2007). The Lord Howe Island stick insect: An example of the benefits of captive management. *The Victorian Naturalist, 124(4)*, 258–261.

Honan, P. (2008). Notes on the biology, captive management and conservation status of the Lord Howe Island Stick Insect (*Dryococelus australis*) (Phasmatodea). *Journal of Insect Conservation, 12(3)*, 399–413. https://doi.org/10.1007/s10841-008-9162-5

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal, 50(3),* 346–363. https://doi.org/10.1002/bimj.200810425

IUCN (2021). The IUCN Red List of Threatened Species (Version 2021-1). International Union for Conservation of Nature and Natural Resources.

IUCN/SSC (2013). Guidelines for reintroductions and other conservation translocations (Version 1.0). IUCN Species Survival Commission.

Jakob-Hoff, R., Harley, D., Magrath, M., Lancaster, M., & Kuchling, G. (2015). Advances in the contribution of zoos to reintroduction programs. In D. P. Armstrong, M. W. Hayward, D. Moro & P. J. Seddon (Eds.), *Advances in reintroduction biology of Australian and New Zealand fauna* (pp. 201–216). CSIRO Publishing.

Jander, U., & Jander, R. (2002). Allometry and resolution of bee eyes (Apoidea). *Arthropod Structure & Development, 30(3)*, 179-193. https://doi.org/10.1016/S1467-8039(01)00035-4.

Joron, M., & Brakefield, P. M. (2003). Captivity masks inbreeding effects on male mating success in butterflies. *Nature, 424*, 191–194. https://doi.org/10.1038/nature01713

Kraaijeveld-Smit, F. J. L., Griffiths, R. A., Moore, R. D., & Beebee, T. J. C. (2006). Captive breeding and the fitness of reintroduced species: a test of the responses to predators in a threatened amphibian. *Journal of Applied Ecology, 43(2)*, 360-365. doi: 10.1111/j.1365-2664.2006.01137.x

Lacy, R. C. (1987). Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. *Conservation Biology, 1(2)*, 143–158. https://doi.org/10.2307/2385830

Land, M. F. (1997). Visual acuity in insects. *Annual Review of Entomology, 42(1)*, 147–177. https://doi.org/10.1146/annurev.ento.42.1.147

Lea, A. M. (1916). Notes on the Lord Howe Island phasma, and on an associated longicorn beetle. *Transactions and Proceedings of the Royal Society of South Australia, 40*, 145–147.

Leather, S. R., Basset, Y., & Hawkins, B. A. (2008). Insect conservation: finding the way forward. *Insect Conservation and Diversity, 1(1)*, 67–69. https://doi.org/10.1111/j.1752-4598.2007.00005.x

Levis, N. A., & Pfennig, D. W. (2016). Evaluating 'plasticity-first' evolution in nature: Key criteria and empirical approaches. *Trends in Ecology & Evolution, 31(7)*, 563–574. https://doi.org/10.1016/j.tree.2016.03.012

Lewis, O. T., & Thomas, C. D. (2001). Adaptations to captivity in the butterfly *Pieris brassicae* (L.) and the implications for *ex situ* conservation. *Journal of Insect Conservation, 5(1)*, 55–63. https://doi.org/10.1023/A:1011348716934

Lim, M. L. M., Sodhi, N. S., & Endler, J. A. (2008). Conservation with sense. *Science, 319(5861)*, 281. https://doi.org/10.1126/science.319.5861.281b

Lord Howe Island Board (2020). Rodent Eradication. Lord Howe Island Board.

McGrath, S. J., Cleave, R. J. , Elgar, M. A., Silcocks, S. C., & Magrath, M. J. L. (2017). Determining host plant preferences for the critically endangered Lord Howe Island stick insect (*Dryococelus australis*) to assist reintroduction. *Journal of Insect Conservation, 21(5)*, 791–799. https://doi.org/10.1007/s10841-017-0002-3

Mikheyev, A. S., Zwick, A., Magrath, M. J. L., Grau, M. L., Qiu, L., Su, Y. N., & Yeates, D. (2017). Museum genomics confirms that the Lord Howe Island stick insect survived extinction. *Current Biology, 27(20)*, 3157–3161. https://doi.org/10.1016/j.cub.2017.08.058

Niven, J. E., & Laughlin, S. B. (2008). Energy limitation as a selective pressure on the evolution of sensory systems. *Journal of Experimental Biology, 211(11)*, 1792–1804. https://doi.org/10.1242/jeb.017574

Pearce-Kelly, P., Jones, R., Clarke, D., Walker, C., Atkin, P., & Cunningham, A. A. (1998). The captive rearing of threatened Orthoptera: a comparison of the conservation potential and practical considerations of two species' breeding programmes at the Zoological Society of London. *Journal of Insect Conservation, 2*, 201–210. https://doi.org/10.1023/A:1009643729536

Perl, C. D., & Niven, J. E. (2016). Differential scaling within an insect compound eye. *Biology Letters, 12(3)*. https://doi.org/10.1098/rsbl.2016.0042

Priddel, D., Carlile, N., Humphrey, M., Fellenberg, S., & Hiscox, D. (2003). Rediscovery of the 'extinct' Lord Howe Island stick-insect (*Dryococelus australis* (Montrouzier)) (Phasmatodea) and recommendations for its conservation. *Biodiversity & Conservation, 12(7)*, 1391–1403. https://doi.org/10.1023/a:1023625710011

Pyke, G. H., & Ehrlich, P. R. (2010). Biological collections and ecological/environmental research: a review, some observations and a look to the future. *Biological Reviews, 85(2)*, 247–266. doi: 10.1111/j.1469-185X.2009.00098.x

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J. Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature methods, 9(7)*, 676–682. https://doi.org/10.1038/nmeth.2019

Seddon, P. J., Armstrong, D. P., & Maloney, R. F. (2007). Developing the science of reintroduction biology. *Conservation Biology, 21(2)*, 303–312. https://doi.org/10.1111/j.1523-1739.2006.00627.x

Slifer, E. H. (1966). Sense organs on the antennal flagellum of a walkingstick *Carausius morosus* Brünner (Phasmida). *Journal of Morphology, 120(2)*, 189–201. https://doi.org/10.1002/jmor.1051200205

Spaethe, J., Brockmann, A., Halbig, C., & Tautz, J. (2007). Size determines antennal sensitivity and behavioral threshold to odors in bumblebee workers. *Naturwissenschaften, 94(9)*, 733–739. https://doi.org/10.1007/s00114-007-0251-1

Stringer, I. A. N., & Chappell, R. (2008). Possible rescue from extinction: transfer of a rare New Zealand tusked weta to islands in the Mercury group. *Journal of Insect Conservation, 12(3)*, 371-382. https://doi.org/10.1007/s10841-008-9149-2

Sutherland, W. J. (1998). Managing habitats and species. In W. J. Sutherland (Ed.), *Conservation Science and Action* (pp. 202–219). Blackwell Science.

Warrant, E. J. (2017). The remarkable visual capacities of nocturnal insects: vision at the limits with small eyes and tiny brains. *Philosophical Transactions of the Royal Society B: Biological Sciences, 372(1717)*. https://doi.org/10.1098/rstb.2016.0063.

Weeks, A. R., Moro, D., Thavornkanlapachai, R., Taylor, H. R., White, N. E., Weiser, E. L., & Heinze, D. (2015). Conserving and enhancing genetic diversity in translocation prgrams. In D. P. Armstrong, M. W. Hayward, D. Moro & P. J. Seddon (Eds.), *Advances in reintroduction biology of Australian and New Zealand fauna* (pp. 127–140). CSIRO Publishing.

Williams, S. E., & Hoffman, E. A. (2009). Minimizing genetic adaptation in captive breeding programs: A review. *Biological Conservation, 142(11)*, 2388–2400. https://doi.org/10.1016/j.biocon.2009.05.034

Wittwer, B., Hefetz, A., Simon, T., Murphy, L. E. K., Elgar, M. A., Pierce, N. E., & Kocher, S. D. (2017). Solitary bees reduce investment in communication compared with their social relatives. *Proceedings of the National Academy of Sciences, 114(25)*, 6569–6574. https://doi.org/10.1073/pnas.1620780114

Woodworth, L. M., Montgomery, M. E., Briscoe, D. A., & Frankham, R. (2002). Rapid genetic deterioration in captive populations: Causes and conservation implications. *Conservation Genetics, 3(3)*, 277-288. https://doi.org/10.1023/A:1019954801089

Zill, S. N., Büschges, A., & Schmitz, J. (2011). Encoding of force increases and decreases by tibial campaniform sensilla in the stick insect, *Carausius morosus*. *Journal of Comparative Physiology A, 197(8)*, 851-867. https://doi.org/10.1007/s00359-011-0647-4

Table 1. Ordinary least squares models for the densities of types of antennal sensilla for which population or sex did not explain a significant amount of variation.

Model/parameter	**Statistics**		
8th antennomer chemoreceptors			
Fixed effects	***df***	***F ratio***	***p > F***
Population (LHI, BP, MZ gen 8–10, MZ gen 14)	3,28	0.226	0.878
Sex (female, male)	1,28	2.370	0.135
Femur length	1,28	0.149	0.702
Apical antennomer tactile hairs			

Fixed effects	***df***	***F ratio***	***p > F***
Population	3,30	0.038	0.990
Sex	1,30	0.003	0.954
Femur length	1,30	0.168	0.685

8th antennomer tactile hairs

Fixed effects	***df***	***F ratio***	***p > F***
Population	3,28	0.692	0.565
Sex	1,28	0.512	0.480
Femur length	1,28	0.893	0.353

Ln(Apical antennomer sensilla campaniforma)

Fixed effects	***df***	***F ratio***	***p > F***
Population	3,30	1.04	0.389
Sex	1,30	0.567	0.457
Femur length	1,30	2.93	0.097

Ln(8th antennomer sensilla campaniforma)

Fixed effects	***df***	***F ratio***	***p > F***
Population	3,28	1.890	0.154
Sex	1,28	0.067	0.798
Femur length	1,28	0.211	0.650

Apical antennomer sensory pores

Fixed effects	***df***	***F ratio***	***p > F***
Population	3,30	0.075	0.973
Sex	1,30	0.140	0.711
Femur length	1,30	0.667	0.421

8th antennomer sensory pores

Fixed effects	***df***	***F ratio***	***p > F***
Population	3,28	2.625	0.070
Sex	1,28	0.151	0.700

Femur length	1,28	4.273	0.048

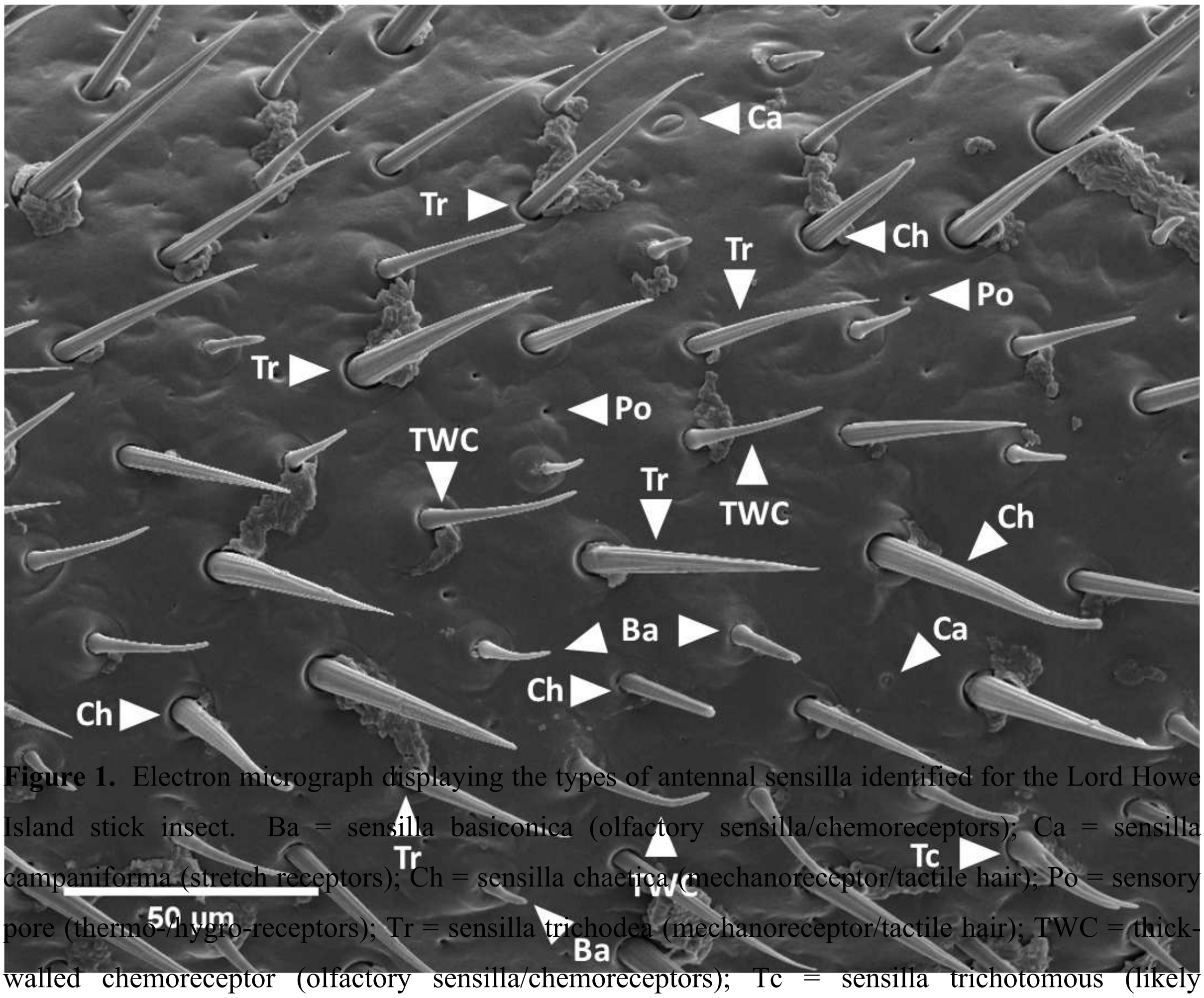


Figure 1. Electron micrograph displaying the types of antennal sensilla identified for the Lord Howe Island stick insect. Ba = sensilla basiconica (olfactory sensilla/chemoreceptors); Ca = sensilla campaniforma (stretch receptors); Ch = sensilla chaetica (mechanoreceptor/tactile hair); Po = sensory pore (thermo-/hygro-receptors); Tr = sensilla trichodea (mechanoreceptor/tactile hair); TWC = thick-walled chemoreceptor (olfactory sensilla/chemoreceptors); Tc = sensilla trichotomous (likely olfactory sensilla/chemoreceptors).

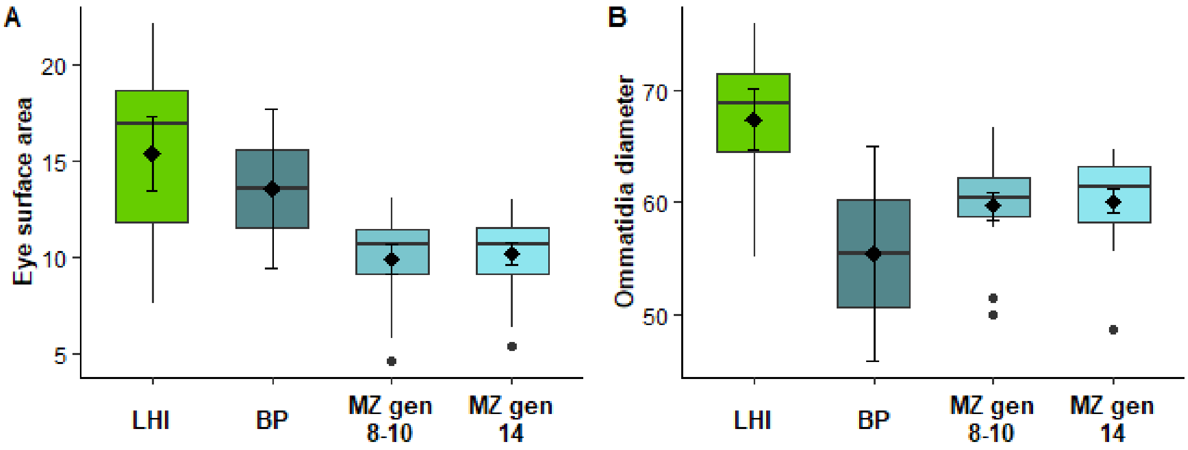
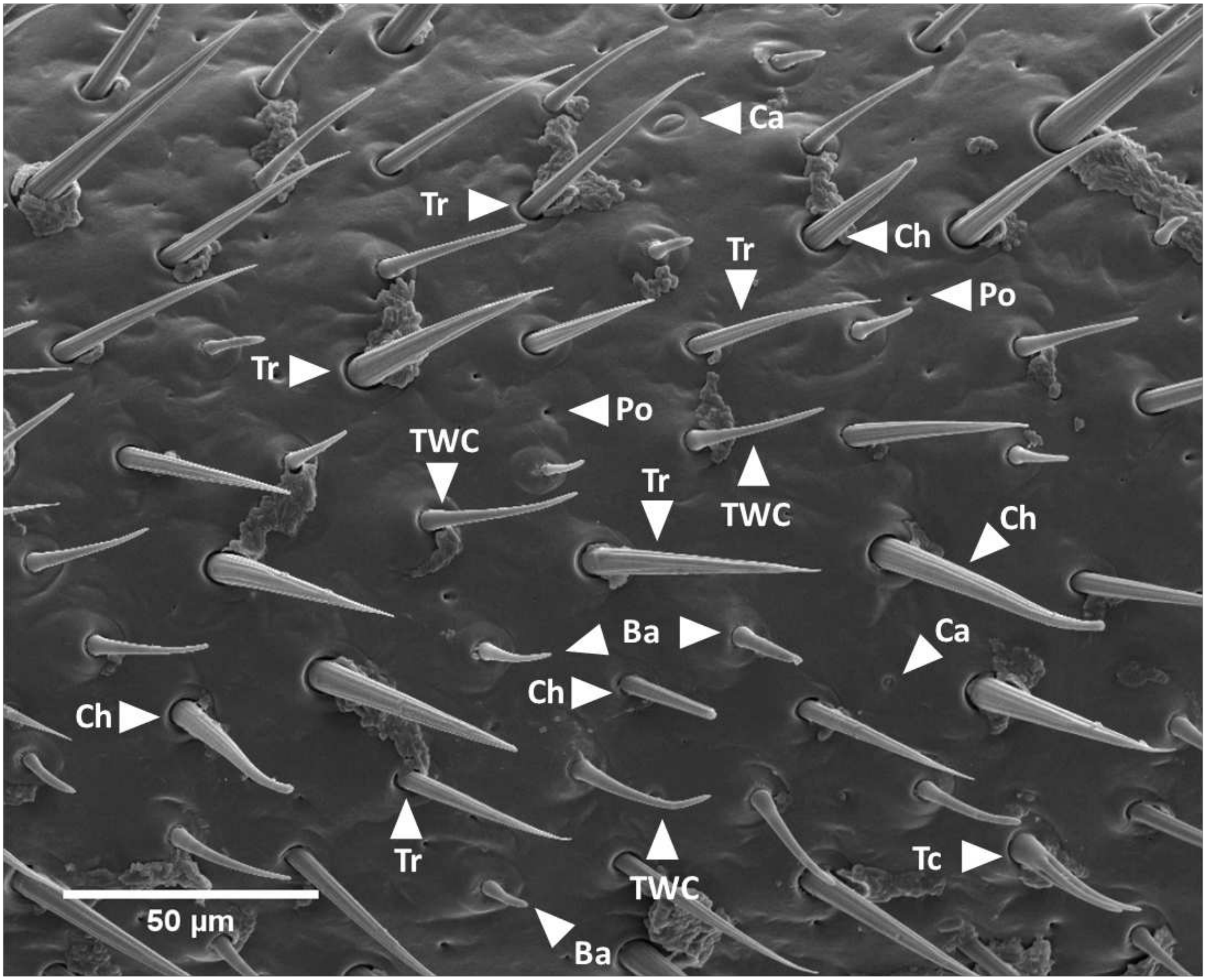


Figure 2. Comparison of compound eye and antennal micromorphology between populations of the Lord Howe Island stick insect. Tails indicate the range; box indicates the interquartile range; horizontal line within the box indicates the median; black diamonds indicate the mean; black capped error bars indicate standard error of the mean; black filled circles represent outliers. Sensilla densities were calculated as the number of sensilla per mm^2 of antenna. **A.** Individuals from the LHI had larger compound eyes by surface area (mm^2) than the captive MZ generation 14. **B.** Individuals from LHI had significantly larger ommatidia (µm) than the wild BP and captive MZ generation 14 populations. **C.** Individuals from LHI had a higher density of antennal chemoreceptors on the apical antennomer compared to the captive bred MZ generation 14; this pattern was driven specifically by **D.** the TWC type of chemoreceptor.



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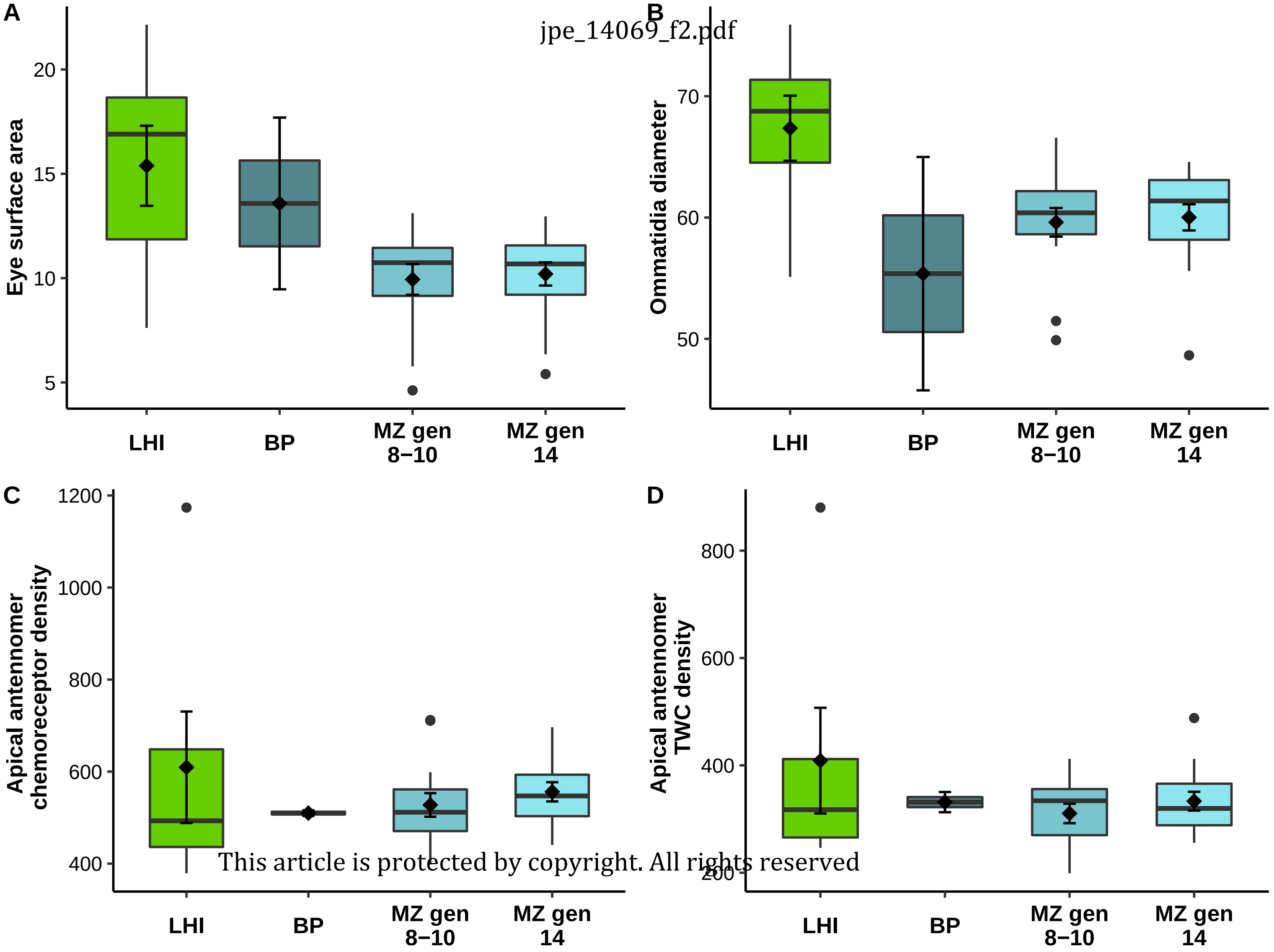
A
Eye surface area
20
15
10
5
LHI
BP
MZ gen 8–10
MZ gen 14
B
Ommatidia diameter
70
60
50
LHI
BP
MZ gen 8–10
MZ gen 14
C
Apical antennomer chemoreceptor density
1200
1000
800
600
400
LHI
BP
MZ gen 8–10
MZ gen 14
D
Apical antennomer TWC density
800
600
400
200
LHI
BP
MZ gen 8–10
MZ gen 14