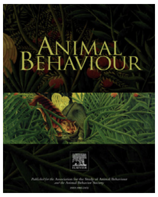
Animal Behaviour 221 (2025) 122961

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav



Special Issue: Tribute to W.G. Eberhard & M.J. West-Eberhard

Mating behaviour of endemic and introduced *Galapaganus* weevils (Coleoptera: Curculionidae) in the Galápagos Islands: linking behavioural mechanism to genomic pattern

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ARTICLE INFO

Article history:
Received 8 September 2023
Initial acceptance 12 May 2024
Final acceptance 29 July 2024
Available online 24 September 2024
MS. number: SI-23-00469R

Keywords:
assortative mating
mate choice
plant-feeding insect
reproductive isolation
speciation

This paper is the first step in a study that aims to use details of mating and courtship behaviour to explain realized patterns of interbreeding between closely related species. We worked with three *Galapaganus* weevil species (Coleoptera: Curculionidae) in the Galápagos Archipelago, one introduced (*Galapaganus howdenae*) and two endemics that are sister species: *Galapaganus conwayensis* and *Galapaganus ashlocki*. We tested three hypotheses regarding divergence in mating behaviour. The first hypothesis is based on the widespread pattern of rapid divergence in sexual traits and predicts pronounced divergence between all three weevil species, with no 'phylogenetic signal'. The second hypothesis is based on the weevils' phylogeny and predicts greater similarity between the endemics relative to the introduced species. The third hypothesis is based on an observation of gene flow into the more distantly related introduced species (*G. howdenae*) from one of the endemics (*G. ashlocki*) and predicts greater overall similarity between the endemic and its more distantly related introduced relative. We found a complex suite of similarities and differences between the weevils. However, we found no predominant pattern of similarity between endemics or between the highland endemic and the introduced species. There was also no broad overall divergence between the three species. These results suggest that realized hybridization and introgression may follow specific key traits that make a disproportionate contribution. We discuss which particular copulatory courtship behaviours may have such a role. Further work involving mate preference and host plant preference tests is required to relate genome-wide patterns of hybridization and introgression between the weevils to potential causes of reproductive isolation between them. It is clear, however, that understanding such patterns will benefit from detailed analysis of potential behavioural causes of reproductive isolation.

Exotic invasive species pose one of the greatest threats to species and habitat diversity today (Wilcove et al., 1998). Invasives bring impacts at the community and ecosystem levels and further pose threats at the genetic level for closely related species. The latter problem may be less visible than ecological change, yet still have major consequences on the health of native populations (Brown et al., 2001; Fitzpatrick & Shaffer, 2007; Rhymer & Simberloff, 1996). Introductions may cause the breakdown of reproductive isolation between invasives and closely related native or endemic species. As gene pools mix during hybridization, population genotypic distinctiveness may be lost. Endemic species are usually rare and may be placed at further risk by hybridization. Hybridization between non-native and native subspecies is known to have caused native species to lose their identity by gene flow and become extinct (Jensen et al., 2005). Hybridization can also lead to introgression and threaten rare parent populations (Rhymer & Simberloff, 1996). The presence of closely related invasive species can threaten endemics even if gene pools do not mix, because the allocation of mating resources to partners from another species (e.g. mating attempts) may reduce the rare endemic species' chances to maintain healthy population sizes (D'Amore et al., 2009; Gröning & Hochkirch, 2008; Rhymer & Simberloff, 1996). Additionally, hybridization with local endemics may promote invasiveness in the introduced populations by increasing genetic variability, creating favourable gene combinations and masking deleterious alleles (Currat et al., 2008; Ellstrand & Schierenbeck,

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https://doi.org/10.1016/j.anbehav.2024.08.011
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2000; Schierenbeck & Ellstrand, 2009). Understanding the mechanisms that determine the nature and outcome of reproductive interactions between invasives and closely related native species is, therefore, crucial for understanding the patterns of hybridization and introgression that may arise, as well as for curving the genetic impact of biological invasions and ensuring the survival of rare native and endemic species.

This paper is the first step in a study that aims to link potential causes of reproductive isolation and realized patterns of hybridization and introgression. This study arose from a collaboration between an evolutionary geneticist (A.S.S.) and an animal behaviourist (R.L.R.). We seek to take advantage of an island system that is a classic focus of studies of adaptation and speciation and at the same time subject to critical threats from invasives, the Galápagos Islands. We worked with three closely related *Galapaganus* weevil species (Coleoptera: Curculionidae). The genus *Galapaganus* contains 15 species in the *Femoratus* and *Darwini* species groups. The 13 species in the *Darwini* group are flightless and fairly heavy-bodied, 10 of which are endemic to the islands (Lanteri, 1992) (Fig. 1). A recent island invader from the *Femoratus* group, *Galapaganus howdenae*, has well-developed wings (Lanteri, 2004), which increases its dispersal potential and may impact its hybridizing abilities (Barton & Bengtsson, 1986). *Galapaganus* endemics, like most single-island endemics, have restricted distributions and their survival may depend on maintaining effective reproductive isolation from close relatives.

We studied the introduced weevil and two endemic species that are sister species: *Galapaganus conwayensis* and *Galapaganus ashlocki* (Sequeira, Lanteri, et al., 2008; Sequeira, Sijapati, et al., 2008) (Fig. 1). The introduction of *G. howdenae* into Santa Cruz and its subsequent expansion into the highlands are fairly recent (Mok et al., 2014; Sequeira et al., 2017). This species trio thus allows studying the patterns of hybridization and introgression during the initial periods of invasion and range expansion in a newly established hybrid zone in fine detail. The three weevils share many life-history traits (Lanteri, 1992). Their larvae develop in the soil feeding on host plant root tissue, while adults spend a significant amount of time on their host plants, which is where they mate. The introduced weevil is more vagile and believed to be a generalist feeder, while the two endemics are flightless and feed on endemic host plant species. Besides being a single island endemic, *G. ashlocki* is a highland specialist (Lanteri, 1992), further constraining its suitable habitat to host plants from higher elevations. Despite having been originally described as a lowland endemic in Santa Cruz and three islands (Lanteri, 1992; Peck, 2006), *G. conwayensis* has recently expanded its range towards the highlands in Santa Cruz (Sequeira et al., 2012) and now its distribution overlaps with that of the highland endemic *G. ashlocki*.

In prior work, we explored the colonization and speciation patterns of *Galapaganus* weevils in the Galápagos, confirming a single colonization of the archipelago and sister-group relationship between the two endemics to Santa Cruz Island (*G. conwayensis* – *G.ashlocki*) (Sequeira, Lanteri, et al., 2008; Sequeira, Sijapati, et al., 2008). Our molecular dating places the accidental introduction of *G. howdenae*, now formally listed as invasive (Causton et al., 2006; Peck, 2006), into Santa Cruz from the highlands of Manabi Province in Continental Ecuador around 1930 (Mok et al., 2014; Sequeira et al., 2017). Even though *G. howdenae* is thought to have been introduced into lowland agricultural zones, we have now repeatedly found it outside those boundaries, side by side with their flightless endemic close relatives, *G. conwayensis* and *G. ashlocki* (Fig. 1). Genetic structure and population demographic patterns indicate that populations of the introduced *G. howdenae* across Santa Cruz are genetically connected and undergoing demographic expansion (Mok et al., 2014; Sequeira et al., 2012). We have not found *Galapaganus* adults of intermediate morphology that might suggest hybrid origin between introduced *G. howdenae* and island endemics (Sequeira, Lanteri, et al., 2008). However, preliminary estimates point to interbreeding between the introduced and endemic species, involving unidirectional gene flow from endemic to introduced *Galapaganus*, in particular from *G. ashlocki* to *G. howdenae* (McKenna et al., 2018; Pangburn, 2016).

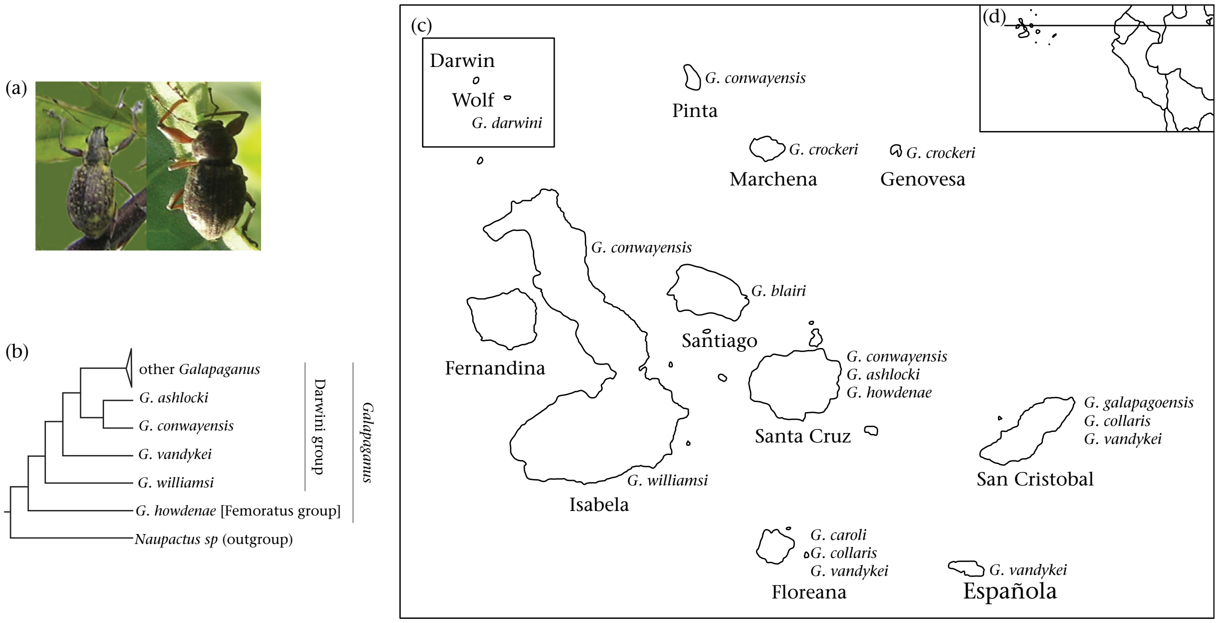

Figure 1. Distribution and phylogenetic relationship of the three *Galapaganus* weevil species we studied. (a) Exemplars of the introduced species *G. howdenae* (left) and the lowland endemic *G. conwayensis* (right) on their respective host plants. (b) Simplified evolutionary relationships of *Galapaganus* species as determined by mitochondrial and nuclear data (adapted from Sequeira, Lanteri, et al., 2008; Sequeira, Sijapati, et al., 2008) with morphologically defined species groups (Lanteri, 1992). (c) Map of the Galápagos archipelago indicating the distribution of *Galapaganus* species. (d) Location of the Galápagos archipelago relative to the Equator and the South American continent.

Our aim is to use details of the behaviour and natural history of the three *Galapaganus* weevils to attempt to explain observed patterns of hybridization and introgression. Causes of reproductive isolation between these species may involve host plant preferences or mate preferences, the latter potentially occurring prior to mating (classical mate choice) or involving processes during or after copulation (cryptic choice) (Coyne & Orr, 2004; Drès & Mallet, 2002; Eberhard, 1985, 1996, 2009; Kirkpatrick & Ravigné, 2002; Kopp et al., 2018; Nosil, 2012; Rosenthal, 2017; Schluter, 2001). We consider other potential causes of reproductive isolation, such as geographical separation or differences in the timing of the mating season (Coyne & Orr, 2004; Drès & Mallet, 2002; Kirkpatrick & Ravigné, 2002; Kopp et al., 2018; Wood & Guttman, 1983; Wood & Keese, 1990), to be less likely to apply to these tropical and (now sympatric) weevils. Here we focus on differences in mating behaviour that may underlie mate choice decisions. In subsequent papers, we will describe patterns of host plant preferences and mate preferences between and within these species.

We tested three hypotheses regarding the form that divergence in mating and courtship behaviour might take. The first hypothesis is based on the widespread pattern of rapid divergence in sexual traits that often produces strong species specificity and contributes to reproductive isolation between diverging populations and species (Andersson, 1994; Arnegard et al., 2010; Boul et al., 2007; Coyne & Orr, 2004; Eberhard, 1985, 1996, 2009; Funk et al., 2009; Mendelson & Shaw, 2005; Safran et al., 2012; Seddon et al., 2013; Svensson et al., 2006; West-Eberhard, 1983, 2014). This hypothesis predicts pronounced divergence between all three weevil species, with no ‘phylogenetic-signal’.

The second hypothesis is based on the observation that, despite the above pattern of rapid divergence and species specificity, some features of mating signals and ornamental displays do carry some phylogenetic signal (e.g. Cocroft & Ryan, 1995; Gingras et al., 2013; Gray, 2022; Prum, 1990). The two endemics are sister species, while the introduced species is more distantly related to both of them (Sequeira, Lanteri, et al., 2008; Sequeira, Sijapati, et al., 2008; Fig. 1). This hypothesis thus predicts greater similarity between the endemics relative to the introduced species. A corollary of this hypothesis would be that speciation in this genus, at least regarding these three species, did not result from divergence in mating behaviour but perhaps from divergence in host plant use (cf. Drès & Mallet, 2002; Kirkpatrick & Ravigné, 2002; Kopp et al., 2018). There is prior support for this hypothesis, as the pattern in genitalia conforms to this prediction: there are no discernible differences in genitalia between the two endemics and differences between them and the introduced species involve subtle (albeit potentially important; see below) details of the apex of the male aedeagus and the size and shape of the female spermatheca (Lanteri, 1992).

The third hypothesis is based on the observation that patterns of hybridization between closely related species may involve asymmetric introgression of ornaments, behaviour and/or mate preferences (e.g. Bennett et al., 2021; Feiner et al., 2024; Rosser et al., 2024; Rossi et al., 2024; Yang et al., 2020). Accordingly, the observed pattern of gene flow into the more distantly related introduced species (*G. howdenae*) from one of the endemics (*G. ashlocki*) (McKenna et al., 2018; Pangburn, 2016) may arise from similarities in mating and courtship behaviour. This hypothesis therefore predicts an asymmetric pattern of greater overall similarity between one endemic (*G. ashlocki*) and its more distantly related introduced relative (*G. howdenae*).

The latter hypothesis may seem to be favoured a priori, as the observed pattern of *G. ashlocki* to *G. howdenae* (McKenna et al., 2018; Pangburn, 2016) is a given that we seek to explain. However, this observation could be explained by host plant preferences or particular mating behaviours having greater relevance than others (see below). Testing the three hypotheses with mating and courtship behaviour contrasts between the weevils will thus help relate behavioural mechanism to observed genetic processes.

To test the hypotheses, we described the mating behaviour of the endemic and introduced *Galapaganus* species. We then contrasted their similarities and differences to observed patterns of hybridization and introgression (McKenna et al., 2018; Pangburn, 2016). We decided which behaviours to include in our descriptions and comparisons on the basis of Eberhard's (1994) criteria for considering behaviours performed during mating to be courtship. These criteria assess the likelihood that a given behaviour may have the function of stimulating the mating partner, rather than merely holding on to the mating partner or being incidental. Namely, the behaviour should be (1) repeated, in general outline, both during a given mating and in different matings, (2) appropriate to produce stimulation of the female (or the male in the case of behaviour by the female), (3) not mechanically necessary for the male to stay physically on or coupled to the female and (4) only performed in the context of mating and not in other contexts suggesting other functions (Eberhard, 1994).

METHODS

We collected adult *Galapaganus* weevils from localities across Santa Cruz Island in the Galápagos during 1–20 March 2022 (Fig. 1). We placed a beating sheet under potential host plants, shook the host plant using a stick and collected the weevils that were dislodged and fell on the sheet. We noted host plant identity at the time of collection. We placed the weevils in 50 ml ventilated conical centrifuge tubes containing host plant material for transport to the laboratory at the Charles Darwin Research Station in Puerto Ayora (~8–10 per tube).

We separated the weevils by species and sex and placed them into transparent rectangular acrylic boxes (L × W × H: 16 × 12 × 7 cm) with ventilation. We provided host plant material as shelter and food and a wet paper towel to maintain humidity (~15–20 weevils per box). We exchanged host plant material and the paper towel every other day. We maintained the boxes on a 12:12 h light:dark cycle at controlled room temperature (24–26 °C). We monitored the weevils every day and removed dead individuals and preserved those in 100% ethanol. We conducted observations for 3 weeks and later deposited voucher specimens in the FCD (Fundación Charles Darwin) entomological collection once we finalized all the mating observations.

Set-up of Mating Observations

To observe matings, we placed five males and five females of the same species in a mating arena. The mating arena consisted of a circular acrylic box 14.5 cm in diameter and 2 cm in height where we provided a small amount of host plant material. We placed the mating arena on an elevated platform that allowed video recording from all angles. Whenever possible, we returned nonmating weevils to their boxes once one male initiated a mating attempt by mounting a female (see below). On occasion, two or three matings were initiated in the arena, in which case we recorded them in parallel with separate video cameras. We later separated and marked each weevil that participated in mating to avoid using them in further trials. In total, we observed 21 mating attempts with *G. howdenae*, 24 mating attempts with *G. conwayensis* and 18 mating attempts with *G. ashlocki*. These resulted in 21 *G. howdenae* matings, 19 *G. conwayensis* matings and 16 *G. ashlocki* matings.

Recording and Storage of Video Footage

We recorded each mating either on a Sony Handycam HDR-CX560, a CX700 or a Kodak PlaySport ZX5 Videocamera. We placed both Sony cameras on Basic (Slik U8000) tripods. We enhanced lighting with two dimmable 5600K USB LED video lights on adjustable tripod stands when needed. We coded the data from each unique mating event recording date, time, box of origin, host plant provided and other observations, and linked those to the video filename. We transferred all video clips to multiple storage platforms (note: A.S.S. conducted all fieldwork and mating trials).

Analysis of Video Footage and Production of Video Logs and Still Images

The four of us independently viewed a random subset of video clips to identify common behaviours for each species. Once a consensus was reached on the set of stereotyped and repeated behaviours related to mating, we produced detailed descriptions for all behaviours. When analysing all clips, we divided the description of each mating between two independent observers. One focused on the front of the weevils (including movements of antennae and legs), the other on the posterior of the weevils (including movement of the genitalia). Each observer used a Python-based data logger (courtesy of Joey Kilmer) to record the occurrence and duration of the different behaviours. We produced short clips displaying each behaviour using iMovie (available as Videos S1−S14). We traced still images of critical moments in those clips using Procreate and Adobe Illustrator to produce illustrations of some behaviours (Fig. 2).

We extracted the proportion of matings with at least one instance of each behaviour, the frequency within a mating (when occurring) and the duration of each behavioural event from the output of the behaviour logger. We defined successful mating as mounting with at least one intromission (see Results). There were some intromissions that were so brief as to suggest they might have constituted ‘pseudocopulations’, but those never occurred in single-intromission matings (see below). We quantified behaviours that occurred multiple times in rapid succession interrupted by periods of inactivity as bouts (male antennae waving, tapping with the tarsi, rubbing with the abdomen, female tap-groom, male genitalic pump; see below). We also recorded the number of elements in each bout (either individual pumps or cycles). Additional details of behavioural analyses are provided in the Appendix.

Statistical Analysis

In addition to describing and comparing the form of the mating behaviours in the three species qualitatively, we also compared their behaviour quantitatively, in terms of the proportion of matings with at least one occurrence for each behaviour (likelihood of occurrence; see Results), how often they occurred within matings (frequency within matings, excluding matings for which the behaviour was not observed) (Appendix, Table A1) and the duration of the behaviours (Appendix, Table A1).

We ran all analyses with JMP Pro 15 (SAS Institute Inc., Cary, NC, U.S.A.). We performed chi-square contingency analysis for the proportions of matings with each behaviour (see Results). We also calculated the mean and SD of occurrence and duration of each behaviour per mating (Appendix, Table A1). We performed

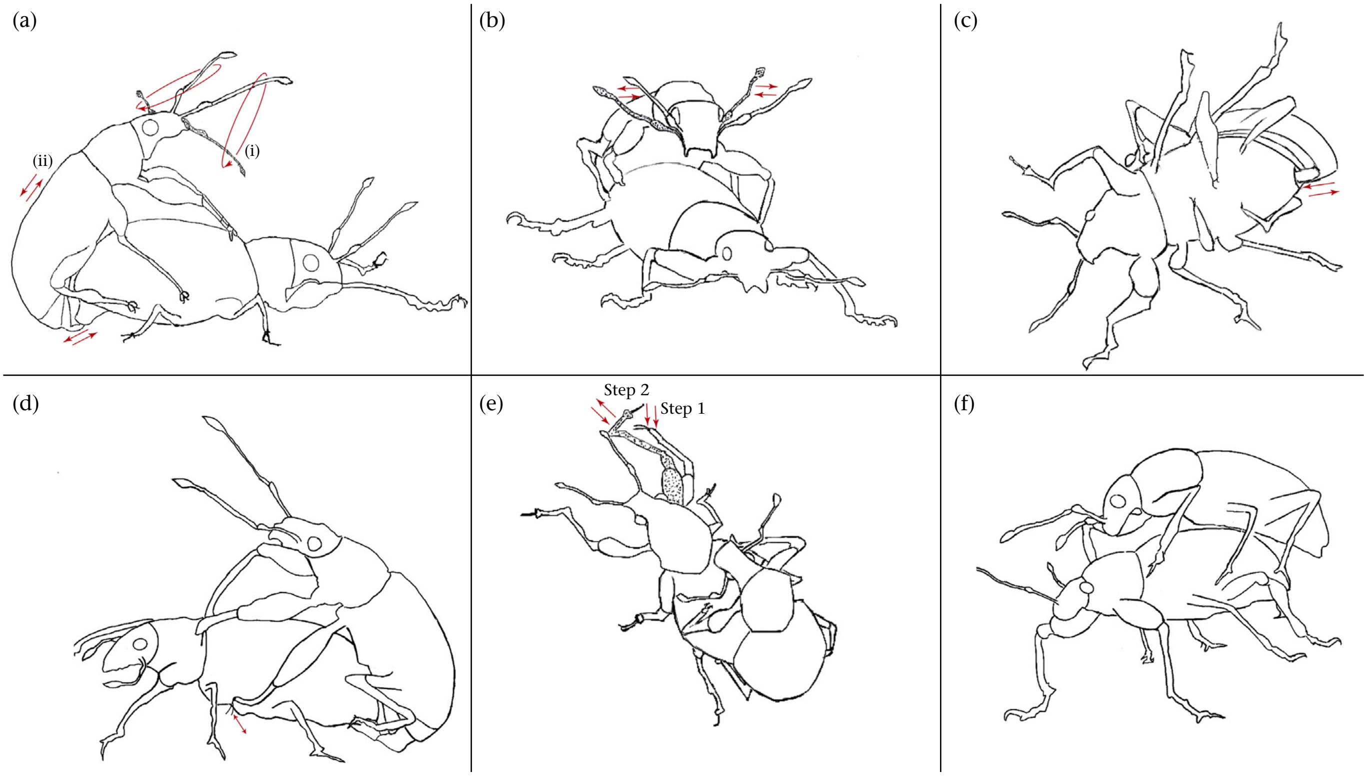

Figure 2. Schematic illustrations of some of the courtship behaviours we described in the three *Galapaganus* species. Red arrows indicate the direction of movement; stippled body parts indicate position after movement. (a) *G. howdenae* pair in intromission, with the male performing: (i) antennae waving, (ii) abdomen rubbing and (iii) genitalic pumping. (b) *G. ashlocki* pair in intromission with the male performing antennae waving. Note the difference in the form of antennae waving between *G. howdenae* (circular movements) and *G. ashlocki* (side-to-side movements). (c) *G. conwayensis* pair in intromission with the male performing genitalic pumping. (d) *G. howdenae* pair in intromission with the male performing tarsal tapping. (e) *G. conwayensis* pair in intromission with the female performing tap-groom. (f) *G. ashlocki* in mate-guarding position.

pairwise nonparametric Wilcoxon comparisons for differences among species pairs in each behaviour (Appendix, Table A1).

For matings that had more than one intromission, we calculated the relative contribution of each intromission's duration as a fraction of the total intromission time. We explored the impact of intromission order and species on relative intromission duration with a two-way ANOVA (Appendix, Fig. A1; Table A2).

We quantified and organized behavioural log data using ‘dplyr’, ‘tidyr’ and ‘forcats’ in RStudio (RStudio Team, 2022). We used jitter plots to illustrate timelines and succession of events produced from the raw data using R packages ‘ggplot2’ and ‘ggridges’ also in RStudio (Appendix, Figs A2—A3).

We tallied instances of similarities between endemics or between one of the endemics and the introduced species (see Results). We only tallied ‘actual’ similarities; e.g. we excluded behaviours that did not differ between any of the species, and when a behaviour was absent in one species, we compared the likelihood of occurrence but not the form or quantitative features. Finally, we used three binomial tests to ask whether, in the final tally, similarities between the endemics or between the highland endemic (*G. ashlocki*) and the introduced *G. howdenae* occurred more often than expected by chance, or whether differences between all three species were prevalent. We assigned a 50% likelihood to any possible similarity/difference.

The above analyses entail many tests with different behaviours, some of which may be correlated with each other, exposing us to the risk of spurious significance (cf. Rice, 1989). However, corrections for multiple tests compromise statistical power (Moran, 2003). We therefore used Moran's (2003) ‘criterion of logic’ whereby a single significant result with a relatively high *P* value in a large table may be spurious, but many significant results likely point to something biologically meaningful. We further note that most of the above tests simply ask whether particular differences were detectable between the species, whereas the comparisons that relate to the hypotheses we test, regarding the form of divergence between the weevils, entail only three tests with the tally of similarities and differences between species, which did not force significance in favour of any one hypothesis (see below).

Ethical Note

All methods were performed in agreement with ASAB/ABS Guidelines for the Use of Animals in Research and institutional laws.

RESULTS

General Description of Galapaganus *Mating Behaviour*

Mating attempts started when a male noticed a female in his vicinity and attempted to mount her without prior courtship. Once mounted on a female, males clasped the side of the female's thorax or abdomen with their legs I and the sides of the female's abdomen with their legs II and II. Males then extruded their aedeagus and attempted genital intromission, which involved moving slightly backward while remaining mounted on the female (Fig. 2). For intromission to succeed, it seemed that females needed to lower the tip of their abdomen and extrude their vulva, although once intromission started the tip of the vulva was no longer visible outside the females' abdomen (Fig. 2a, c, Videos S1—S3). In all three species, the proportion of failed mating attempts (leading to no intromission) was low (*G. howdenae*: 0% of 21 mating attempts; *G conwayensis*: 20% of 24 attempts; *G. ashlocki*: 11% of 18 attempts). Matings could involve one or multiple intromissions.

As our observation trials started with multiple males and females per container, we were able to observe that females of the introduced species (*G. howdenae*) and the highland endemic (*G. ashlocki*) often mated with multiple males; however, each individual only contributed one mating to the data reported here.

Males in the two endemic species (*G. conwayensis* and *G. ashlocki*) sometimes remained on the female after they had withdrawn the aedeagus and attempted no further intromission, which we consider mate guarding (Andersson, 1994; Thornhill & Alcock, 1983). A guarding male's position on the female was somewhat forward compared to that during intromission, the angle almost parallel with the female (Fig. 2f, Videos S4—S5). We noted no species differences in this position. Females being guarded by a male often walked around the arena or fed on a piece of host plant.

Males of all three species dismounted the female at different angles after the completion of intromission (*G. howdenae*) and/or guarding (*G. conwayensis* and *G. ashlocki*). In *G. howdenae*, the male turned 45—90° on the female's back, then walked away. In *G. conwayensis*, the male walked backwards off the female, or turned 90—140° on her and walked away. In *G. ashlocki*, the male turned 180° on the female's back and walked away.

In some cases, we observed a male attempting to dislodge another male that was mating with a female. In four *G. howdenae* matings, the intruder male climbed on the mating pair and the mating male pushed back with his legs (any pair of legs could be involved). On one occasion, a mating *G. howdenae* male interrupted his genitalic pumping when another male approached. On another occasion in *G. howdenae*, the mating male remained inactive in intromission during an attempt to dislodge him. In *G. ashlocki*, intruder males also attempted to dislodge mating males by approaching and mounting the mating pair. In *G. ashlocki*, we observed one intruder male successfully interrupt intromission for two separate mating pairs (once in one mating and thrice in the other mating). On a separate occasion, the *G. ashlocki* mating male remained in guarding position and made jerking motions when the intruding male was close by.

On three occasions in *G. howdenae*, the female dislodged the male from a mounted position by pushing him with her antennae and legs III, then pushing him with her legs I after he had dismounted.

Copulatory Courtship

Males courted the female while mounted, during and out of intromission (but not during mate guarding). Males used one or more of the following behaviours to court the female: waving their antennae over her eyes; tapping the sides of the female's elytra and abdomen with their tarsi II; rubbing the top of the female's elytra with their abdomen; and pumping their aedeagus in and partially out during intromission. Females also performed one movement that could stimulate the male: they tapped the substrate and then groomed their own antennae with their legs I.

Antennae waving

Males of *G. howdenae* and *G. ashlocki* repeatedly waved their antennae above the female's eyes in rhythmic, stereotyped, species-specific ways (Fig. 2a and b). In *G. howdenae*, males waved their antennae in circles, extending them to the side, sweeping them downward and backwards, then up (Video S6). By contrast, *G. ashlocki* males waved their antennae sideways, alternating left and right (Video S7). In most cases, the males' antennae did not contact the females, but the movements would be clearly visible to them. On one occasion in *G. ashlocki*, the antennae appeared to tap the back of the female's thorax.

Males of *G. conwayensis* occasionally moved their antennae during matings, but the movements were not stereotyped and seemed incidental, so we did not consider them courtship.

Tapping the female with tarsi II

Male *G. howdenae* and, with much less frequency male *G. conwayensis*, repeatedly tapped the sides of the female's elytra and abdomen with their tarsi II in a rhythmic and stereotyped way (Fig. 2d, Video S8). We noted no species difference in the form of this behaviour.

Rubbing the female with the abdomen

We only observed this behaviour in the introduced species (*G. howdenae*). Males repeatedly rubbed the top of the female's elytra with their abdomen, which had a row of setae not present in the other species, in a rhythmic and stereotyped way (Figs 2a and 3, Video S9).

We observed a jerking motion reminiscent of the above abdomen rubbing in the lowland endemic (*G. conwayensis*). However, this was sporadic and nonstereotyped and performed inconsistently; we therefore did not consider it courtship.

Genitalic pumping

In all three species, we observed repeated, rhythmic and stereotyped pumping movements of the males' sternite IX and aedeagus during intromission. This brought the aedeagus slightly in and out of the female and moved the female's abdomen slightly up and down (Fig. 2a, Videos S10–S12). We thus considered that this pumping could constitute genitalic courtship. We noted no species difference in the form of this behaviour.

Female tap-groom

Females in both endemic species (*G. conwayensis* and *G. ashlocki*) performed a rhythmic and stereotyped behaviour that we considered could be sensed by a male mounted on them, through contact with their body. The females tapped one of their legs I on the substrate two to three times (step 1 in Fig. 2e), then groomed their antennae once from the scape to the club with the same leg (step 2 in Fig. 2e, Videos S13–S14). Females repeated these tap-groom movements in bouts. We noted no species differences in this movement.

Proportion of Matings with Each Behaviour

We compared the species in terms of the proportion of matings in which the different behaviours occurred at least once (Table 1). The proportion of mating attempts that led to at least one intromission (i.e. the proportion of mating attempts that were successful) differed significantly between the introduced species (*G. howdenae*) and the lowland endemic (*G. conwayensis*), with the former showing the highest likelihood of successful intromission, followed by the highland endemic *G. ashlocki* (Table 1). The lowland endemic (*G. conwayensis*) showed the highest likelihood of multiple failed attempts prior to the first intromission (Table 1). The proportion of matings with multiple mountings and remountings of the same pair as well as the proportion of matings with multiple intromissions were similar in the three species (Table 1).

All five copulatory courtship behaviours (males: genitalic pumping, antennae waving, rubbing with the tarsi, rubbing with the abdomen; females: tap-groom) varied significantly between pairs of species in the likelihood of at least one occurrence (Table 1). Matings with genitalic pumping were more common in the

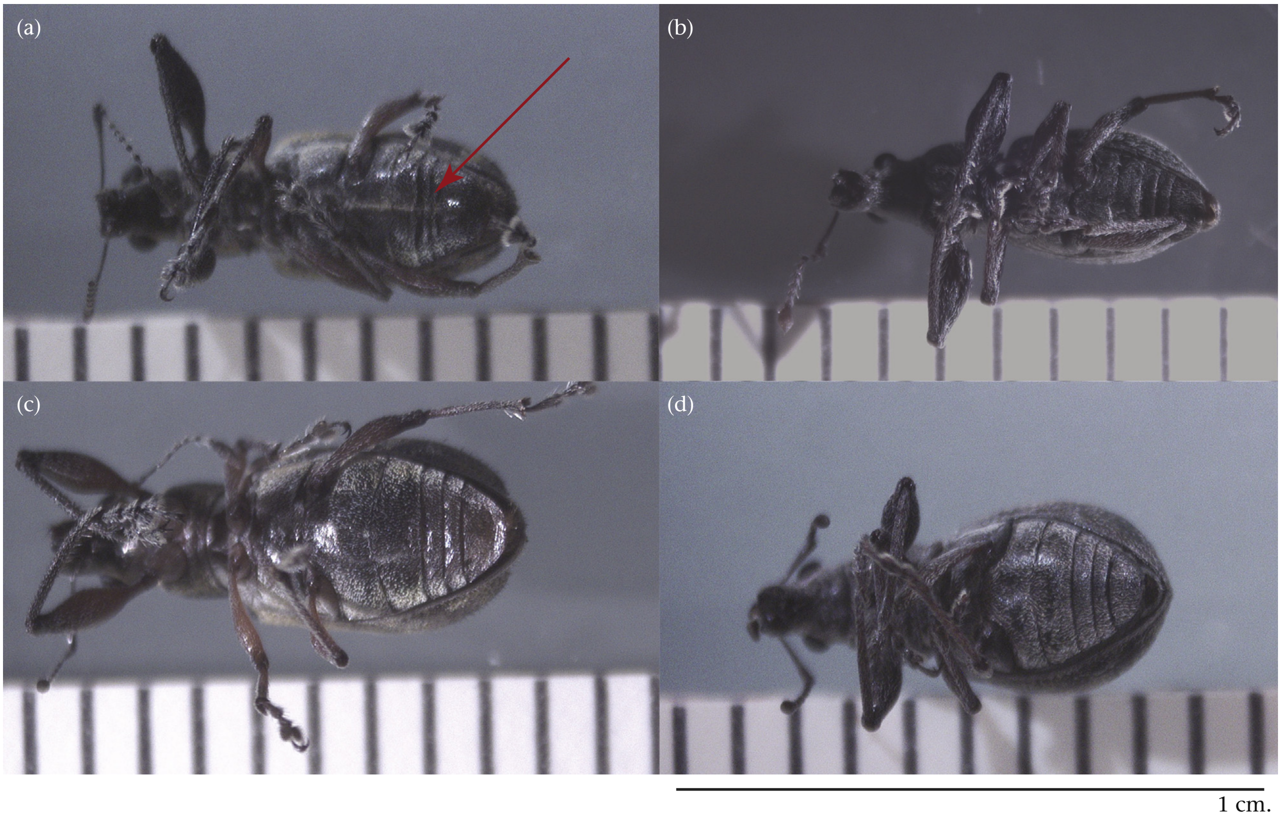

Figure 3. Ventral view of the abdomen in *G. howdenae* and *G. conwayensis*. (a) *G. howdenae* male. (b) *G. conwayensis* male. (c) *G. howdenae* female. (d) *G. conwayensis* female. Arrow indicates the abdominal row of setae in *G. howdenae* males that we hypothesize may be connected to the abdomen-rubbing behaviour present only in that species.

Table 1
Proportion of *Galapaganus* matings in which the different mating behaviours occurred at least once

Proportion of matings with the behaviour	*G. howdenae* (*N*=21 attempts, 21 matings)	*G. conwayensis* (*N*=24 attempts, 19 matings)	*G. ashlocki* (*N*=18 attempts, 16 matings)	Species comparisons: χ^2, *P*
One or more successful intromission attempts	1	0.79	0.89	**h–c: 6.83, 0.009** h–a: 3.2, 0.07 c–a: 0.73, 0.39
Failed intromission attempts before success	0.05	0.21	0	h–c: 2.54, 0.11 h–a: 1.15, 0.282 **c–a: 5.32, 0.02**
Multiple intromissions	0.10	0.26	0.31	h–c: 1.98, 0.158 h–a: 2.81, 0.09 c–a: 0.103, 0.74
Multiple mounting (remounting in same pair after successful mating)	0.05	0.05	0	h–c: 0.05, 0.94 h–a: 1.113, 0.29 c–a: 1.19, 0.27
Male genitalic pump	0.95	0.63	1.00	**h–c: 6.983, 0.0082** h–a: 1.154, 0.282 **c–a: 10.02, 0.0015**
Male antennae waving	1	0	0.83	**h–c: 55.35, <0.0001** h–a: 3.355, 0.067 **c–a: 20.7, <0.0001**
Abdomen rubbing	0.57	0	0	**h–c: 20.187, <0.0001** **h–a: 17.94, <0.0001** c–a: 0, 0
Tarsal tapping	0.57	0.02	0	**h–c: 10.32, 0.0013** **h–a: 17.94, <0.0001** c–a: 2.54, 0.11
Female tap-groom	0	0.63	0.75	**h–c: 41.76, <0.0001** **h–a: 32.53, <0.0001** c–a: 0.479, 0.489
Guarding	0	0.63	0.31	**h–c: 23.86, <0.0001** **h–a: 9.43, 0.0021** c–a: 3.61, 0.057

Proportions were calculated over the total number of observed mating attempts per species or the total number of successful matings, as applicable. We report comparisons for each pair of species conducted with contingency table chi-square tests. For each row, we indicate the species being compared with the first letter of their epithets (e.g. h = *G. howdenae*; c = *G. conwayensis*; a = *Galapaganus ashlocki*). Significant comparisons in bold. With 14 of 33 tests being significant (and an additional 4 being marginally significant, which we did not count), Moran's (2003) criterion suggests a low likelihood of spurious results.

introduced species (*G. howdenae*) and the highland endemic (*G. ashlocki*) than in the lowland endemic (*G. conwayensis*). Antennae waving was common in the introduced species (*G. howdenae*) and the highland endemic (*G. ashlocki*) and absent in the lowland endemic (*G. conwayensis*). Tapping with the tarsi and rubbing with the abdomen was common in the introduced species (*G. howdenae*) and significantly less prevalent or absent in matings of both endemics. Matings with female tap-groom were common in both endemics (*G. conwayensis* and *G. ashlocki*) and absent in the introduced species (*G. howdenae*) (Table 1). Matings with guarding were common in both endemics (*G. conwayensis* and *G. ashlocki*) and absent in the introduced species (*G. howdenae*) (Table 1).

Frequency of Mating Behaviours (within Matings), Duration of Mating Behaviours and Other Details

We also compared the three weevil species in terms of how often the different behaviours occurred in matings in which they occurred at least once and in terms of their duration. Additionally, we described the transitions between behaviours and male female back-and-forth interactions and compared them between the species. We present those results in the Appendix and provide an overall tally below.

Morphological Link to the Behaviour of the Male Rubbing the Female with His Abdomen in G. howdenae

Most *G. howdenae* matings included one or more bouts of the male rubbing the female with his abdomen (Table 1). All of 24 *G. howdenae* males examined had a row of abdominal setae placed centrally from the medial area to the posterior end of the abdomen (Fig. 3a). Such setae were absent in *G. howdenae* females (Fig. 3c) and in both sexes in *G. conwayensis* (Fig. 3b, d) and *G ashlocki* (not shown). The area of the male's abdomen with the row of setae is placed such that it would come in contact with the posterior end of the female's elytra during intromission (Fig. 2a) and would likely be involved in stimulating the female when the male rubs her with his abdomen. Moreover, *G. howdenae* females have particularly long setae in their elytral declivity, an area of likely contact with the male's abdominal row of setae (A. Lanteri, personal communication).

Tally of Species Similarities and Differences

We found a complex suite of similarities and differences between the endemic species and between the endemic species and the introduced species (Table 2). Matings in all species started similarly, when the male noticed the female, then mounted her and attempted intromission. All three species shared most of the features of intromissions, except their duration. They were also similar in the form and duration of male genitalic pumping.

We found several differences in broad, qualitative features of mating behaviour (Table 2). There were intriguing similarities between the introduced species and the highland endemic. Males of *G. howdenae* and *G. ashlocki* were more likely to perform genital pumping (and performed more bouts of pumping) than males of *G. conwayensis*. Males of these two species also courted the female by waving their antennae over the female's eyes, which males of *G. conwayensis* did not do. The form of this waving differed between *G. howdenae* and *G. ashlocki*, however. Conversely, males of the introduced species (*G. howdenae*) and the other endemic species

Table 2
Tally of qualitative and quantitative behavioural differences between the three *Galapaganus* weevils

Behaviour	Measure (source of data in contrast)	*G. howdenae*	*G. conwayensis*	*G. ashlocki*	Species comparisons		
					c–a	h–a	h–c–a
Mating	Average duration (TA1, L1)	○	●	●	✓	✘	✘
Intromission	Proportion of matings with at least one (T1, L1)	●	○	○	✓	✓	✘
				●			
	Form	●	●	●	–	–	–
	Proportion of matings with multiple (T1, L3)	●	●	●	–	–	–
	Average number of events per mating (TA1, L5)	●	●	●	–	–	–
	Average number of events beyond the first (TA1, L6)	●	●	●	–	–	–
	Average duration (TA1, L7)	○	○	●	✘	✘	✘
Male genitalic pump (bouts)	Proportion of matings with at least one (T1, L6)	●	○	●	✘	✓	✘
	Form	●	●	●	–	–	–
	Average number of bouts per mating (TA1, L9)	●	○	○	✓	✓	✘
				●			
	Average duration (TA1, L11)	●	●	●	–	–	–
Male antennae waving (bouts)	Proportion of matings with at least one (T1, L7)	●	○	●	✘	✓	✘
	Form	●	NA	○	–	✘	–
	Average number of bouts per mating (TA1, L12)	●	NA	●	–	✓	–
	Average duration (TA1, L14)	●	NA	●	–	✓	–
Abdomen rubbing (bouts)	Proportion of matings with at least one (T1, L9)	●	○	○	✓	✘	✘
	Form	●	NA	NA	–	–	–
	Average number of bouts per mating (TA1, L21)	●	NA	NA	–	–	–
	Average duration (TA1, L23)	●	NA	NA	–	–	–
Tarsal tapping (bouts)	Proportion of matings with at least one (T1, L10)[a]	●	◍	○	✘	✘	✓[a]
	Form	●	●	NA	–	–	–
	Average number of bouts per mating (TA1, L18)	○	●	NA	–	–	–
	Average duration (TA1, L20)	●	●	NA	–	–	–
Female tap-groom (bouts)	Proportion of matings with at least one (T1, L8)	○	●	●	✓	✘	✘
	Form	NA	●	●	✓	–	–
	Coordination with male courtship	NA	○	●	✘	–	–
	Average number of bouts per mating (TA1, L15)	NA	●	●	✓	–	–
	Average duration (TA1, L16)	NA	●	●	✓	–	–
Guarding	Proportion of matings with one (T1, L11)	○	●	●	✓	✘	✘
	Form	NA	●	●	–	–	–
	Average duration (TA1, L24)	NA	●	●	✓	–	–

Species being compared are indicated with the first letter of their epithets (e.g. h = *G. howdenae*; c = *G. conwayensis*; a = *Galapaganus ashlocki*). Identical symbols represent similarities between species. Open circles identify lower values; solid black circles identify higher values; the grey circle identifies an intermediate value. NA: indicates that the comparison is not possible given that the behaviour is absent in that species. Dashes denote comparisons that were excluded from the final tally because we did not consider them to involve 'actual' differences (see text for rationale).

[a] Counted as a difference despite the comparison being nonsignificant because it involved a low likelihood of occurrence (2%) versus an absolute absence (see text).

(*G. conwayensis*) tapped the female with their tarsi II, which males of *G. ashlocki* did not do. Only *G. howdenae* males rubbed the female with their abdomen, where a row of ventral setae (exclusive to males of this species; Fig. 3) may be involved in stimulating the female. Males in both endemics (*G. conwayensis* and *G. ashlocki*) performed mate guarding, which males of *G. howdenae* did not do.

Females of *G. conwayensis* and *G. ashlocki* performed a tap-groom behaviour that females of *G. howdenae* did not. In *G. ashlocki*, this behaviour appeared to occur in alternation with male antenna waving in approximately one-third of the matings in which both behaviours co-occurred (Appendix, Fig. A3).

We also found differences in quantitative details of the weevils' mating behaviour (Table 2). The endemic species (*G. ashlocki* and *G. conwayensis*) had similar overall mating duration and differed in this feature from the introduced species. The endemics were also similar in the duration of bouts of female tap-groom and in the duration of guarding.

The main differences between the endemics and the introduced species were the overall duration of matings (longer in the endemics), the likelihood of successful matings (with at least one intromission) (higher in *G. howdenae* than in *G. conwayensis*), the duration of intromissions (longer in the highland endemic *G. ashlocki* than in *G. howdenae*), the likelihood and number of bouts of male genitalic pumping (higher in the introduced *G. howdenae* than in the lowland endemic *G. conwayensis*), the likelihood of male antennae waving (always in the introduced *G. howdenae* and absent in the lowland endemic *G. conwayensis*), the likelihood of tarsal tapping and abdomen rubbing (exclusive or almost exclusive to *G. howdenae*), the likelihood of female tap-groom (common in both *G. conwayensis* and *G. ashlocki*, absent in *G. howdenae* and possibly coordinated with male antennae waving only in *G. ashlocki*).

Tallying up the above comparisons, the two endemics (*G. conwayensis* and *G. ashlocki*) were more similar to each other than to the introduced *G. howdenae* in 10 out of a possible 15 traits (one-tailed binomial test: $P = 0.15$; Table 2). There was only one trait out of a possible 10 (the proportion of matings with male tarsal tapping; Table 2) that could be said to differ between all three species ($P = 0.013$) and that required counting the nonsignificant comparison of 2% versus 0% proportions between the endemics (Table 1); otherwise there was no such trait. The introduced *G. howdenae* was more similar to the highland endemic *G. ashlocki* in six out of possible 13 traits ($P = 0.50$; Table 2).

DISCUSSION

We described the mating behaviour of three closely related *Galapaganus* weevils on Santa Cruz Island, Galápagos, two endemic and one introduced species. Our goal was to identify the behavioural mechanisms behind the observed introgression from the highland endemic species (*G. ashlocki*) into the introduced species (*G. howdenae*) (McKenna et al., 2018; Pangburn, 2016). Of particular conservation concern is the ability of *G. ashlocki* to maintain viable population sizes and species boundaries, as it is a single-island endemic and highland specialist. Additionally, *G. conwayensis* may also be at risk, despite also being found in two other neighbouring islands (Northern Isabela and Pinta) (Sequeira, Sijapati, et al., 2008). We were also interested in whether the invasion success of *G. howdenae* may involve adaptive traits acquired from local endemics.

We tested three relatively ‘naïve’ hypotheses about the form that divergence in mating behaviour might take between the three weevils. On the basis of basic sexual selection theory (West-Eberhard, 1983, 2014), one hypothesis posits that all three species should be broadly divergent. Another possibility is that species similarities may follow phylogenetic relatedness (Sequeira, Lanteri, et al., 2008; Sequeira, Sijapati, et al., 2008), with the two endemics being most similar. The third hypothesis is based on the observation of introgression (McKenna et al., 2018; Pangburn, 2016) and states that the highland endemic and the introduced species should be most similar overall.

We found a complex suite of similarities and differences between the weevils (Table 2). Any of these could play a role in precopulatory mate choice (i.e. influence whether intromission occurs; Andersson, 1994; Rosenthal, 2017). Furthermore, the continuation of courtship behaviours throughout mating suggests they may also influence cryptic female choice (Eberhard, 1985, 1996, 2009). There was, however, no predominant pattern of overall similarity between endemics (expected on phylogenetic grounds) or between the highland endemic and the introduced species (expected on the grounds of the observed introgression). There was also no broad overall divergence between the three species (in fact, such cases were rarer than expected by chance).

These results suggest that realized hybridization and introgression may not follow any one particular pattern of overall similarity or difference in mating and courtship behaviours. Instead, a few particular key traits may make a disproportionate contribution. This represents a modified version of the third hypothesis above, with the observed asymmetric introgression involving particular aspects of mating behaviour or mate preferences (cf. Bennett et al., 2021; Feiner et al., 2024; Rosser et al., 2024; Rossi et al., 2024). In the case of the weevils, such traits may include the likelihood and number of bouts of genital pumping, courtship by waving the antennae above the females' eyes, lack of rubbing the female with the abdomen, the occurrence of female tap-groom and its quantitative features and the occurrence of mate guarding (Table 2). Perhaps females from the introduced *G. howdenae* more readily accept *G. ashlocki* males due to their performance of antennae waving (albeit differing in form) or their similarly active genitalic pumping? Further work will be required to assess this possibility. For example, ablation experiments could test the role of the performance and form of antennae waving for mate choice and cryptic mate choice. Other possibilities that will also require testing include male *G. ashlocki* being overall more likely to attempt mating with female *G. howdenae* than in other species combinations.

The relative paucity of differences in the mating and courtship behaviours of the two endemics (Table 2), combined with their similarities in genitalic morphology (Lanteri, 1992), suggests that reproductive isolation between these close relatives may hinge on habitat and host plant preferences (Kopp et al., 2018). Such barriers may be breaking up, however, as all three species now overlap in the highlands of Santa Cruz where they all have been found feeding on the same host plants (Sequeira et al., 2012). As above, however, the actual outcome may depend on a few specific traits; e.g. the presence of courtship with the male antennae in *G. ashlocki* and its absence in *G. conwayensis*.

Functional Considerations and Future Directions

In all three *Galapaganus* species, the second intromission in matings with multiple intromissions was proportionally longer than the other intromissions (Appendix, Fig. A1) and similar in absolute duration to the intromission in single-intromission matings. This suggests that second intromissions in multiple-intromission matings may be functionally equivalent to the intromission in single-intromission matings, regarding when the transfer of sperm and/or seminal fluids occurs. The function of the shorter subsequent intromissions after the longer second intromission is less clear. Possible functions may include stimulating the female to influence cryptic choice (Eberhard, 1994, 1996, 2009, 2015), further transfer of sperm or seminal substances or a form of ‘premate guarding’ (cf. Sillén-Tullberg, 1981; Tadler et al., 1999) with potential stimulation, although then the function of the quite prevalent subsequent longer guarding period in both endemic species would be harder to understand.

We found that *G. howdenae* males rubbed the female with their abdomen, where a row of ventral setae may stimulate the female. Such stimulation may involve touch and/or the transfer of a pheromone (cf. Faustini et al., 1982; Faustini & Halstead, 1982). It will be interesting to test the role of these sensory modalities in the effectiveness of courtship in future work.

Females in *G. conwayensis* and *G. ashlocki* performed a tap-groom behaviour, which in *G. ashlocki* sometimes occurred in alternation with male antenna waving (Appendix, Fig A3). This suggests the possibility of some back-and-forth stimulation between males and females; e.g. as with ‘copulatory dialogues’ (Peretti et al., 2006; Rodríguez, 2015). This is another possibility for a key trait that may determine the likelihood and sign of introgression between species.

Conclusions

Further work involving mate preference and host plant preference tests is required to relate genome-wide patterns of hybridization and introgression between the weevils to potential causes of reproductive isolation between them. It is clear, however, that such patterns arise not from simplistic overall similarities or differences in mating behaviours, but from key shared or distinguishing features. We suggest that our understanding of reproductive isolation and assortative mating (Coyne & Orr, 2004; Kirkpatrick & Ravigné, 2002; Kopp et al., 2018) will profit from relating the form of potential mechanisms to realized genomic patterns of hybridization and introgression. Such progress may ideally be conducted by naturalist–geneticist collaborations that straddle across fields of inquiry (see West-Eberhard, 2025).

Author Contributions

Gabriella Karcz: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. **Andrea S. Sequeira:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Rafael L Rodríguez:** Conceptualization, Formal analysis, Methodology, Validation, Writing – original draft, Writing – review & editing. **Misheel Amarsanaa:** Data curation, Formal analysis, Investigation, Visualization, Writing – original draft.

Data Availability

Data for this study are available as Supplementary Material.

Declaration of Interest

The authors declare no conflicts of interest.

Acknowledgments

We thank the Galapagos National Park Directorate (GNPD) and the Charles Darwin Foundation (CDF) for their support of this project, as well as Heinke Jäger, Jacqueline Rodríguez and Karen Vera for their invaluable input and support throughout the research process and Roberto Ballesteros for his guidance and assistance in the field. Their insights and expertise were instrumental in shaping the direction of this project. This research was performed under research permit PC-18-21, issued by the GNPD. This publication is contribution number 2690 of the Charles Darwin Foundation for the Galápagos Islands. Funds endowed to Wellesley College by Gordon and Althea Lang '26 supported A.S.S. during the travelling and field portions of this project. Partial support during the writing of the manuscript came from U.S. National Science Foundation (NSF) grant IOS–1855962 to R.L.R. and C. Desjonquères.

Supplementary Material

Supplementary material associated with this article is available at https://doi.org/10.1016/j.anbehav.2024.08.011.

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Appendix

Frequency of Behaviours within Matings

We also compared the species in terms of how often the different behaviours occurred in matings where they occurred at least once (Table A1). The two endemics showed similar numbers of failed intromission attempts before a successful one and of repeat mountings after a successful mating. The number of intromissions and intromissions beyond the first (or repeat) were similar in the three species (Table A1).

The number of bouts of male genitalic pumping was highest in the highland endemic (*G. ashlocki*), slightly lower in the introduced species (*G howdenae*) and lowest in the lowland endemic (*G conwayensis*); this difference was significant only between the latter two species (introduced and the lowland endemic). The number of bouts of antennae waving was similar in the highland endemic (*G. ashlocki*) and the introduced species (*G. howdenae*). One behaviour that was almost exclusive to the introduced species (*G. howdenae*) (tapping with the tarsi II; Table 1) differed significantly in frequency compared with one of the lowland endemics (*G. conwayensis*). Female tap-groom (exclusive to the endemics) occurred at similar frequencies in the two species (*G. conwayensis* and *G. ashlocki*) (Table A1).

Table A1

Quantitative comparison of the mating behaviour of the three *Galapaganus* species

Behaviour	Measure	Mean±SD			Species comparisons: *Z*, *P*
		G. howdenae	*G. conwayensis*	*G. ashlocki*	
Mating	Duration (min)	12.3±11.4	68.1±142.6	44.7±48.6	**h–c: 2.58, 0.031** **h–a: 2.15, 0.009** c–a: 0.37, 0.71
Repeated mounting after successful mating (same pair)	No. of mountings	2±0	2±0	N/A	h–c: 0.00, 1
	Duration (min)	0.7±0.5	46.57±31.19	N/A	h–c: 1.16, 0.2
Failed intromission attempts before success	No. of attempts	1±0	4±5.35	N/A	h–c: −0.39, 0.69
Intromission	No. of intromissions	1.1±0.5	1.7±1.4	1.4±0.9	h–c: −1.4, 0.14 h–a: −1.24, 0.21 c–a: 0.21, 0.82
	No. of intromissions beyond the first	1.5±0.7	2.8±1.3	2±0.8	h–c: −1.28, 0.19 h–a: −0.5, 0.61 c–a: 0.81, 0.41

(*continued on next page*)

Table A1 (*continued*)

Behaviour	Measure	Mean±SD			Species comparisons: *Z*, *P*
		G. howdenae	*G. conwayensis*	*G. ashlocki*	
	Duration (min)	11.2±11.6	17.9±4.1	34.48±28.2	h–c: 1.22, 0.22 **h–a: 2.62, 0.001** c–a: −1.27, 0.20
Between-intromission interval	Duration (min)	0.1±0	32.4±25	7.9±11.1	h–c: 1.17, 0.24 h–a: 1.06, 0.28 c–a: 1.34, 0.17
Male genitalic pump	No. of bouts	7.3±6.9	2.2±3.38	8.1±10.16	**h–c: 2.41 (0.015)** h–a: 0.64 (0.52) c–a: −1.07 (0.28)
	Pumps/bout	1.9±1.6	1.7±2.21	1.8±2.8	
	Bout duration (min)	0.43±0.35	0.55±0.66	1.06±1.53	h–c: −1.19 (0.23) h–a: 1.51 (0.13) c–a: −0.97 (0.23)
Male antennae waving	No. of bouts	6.14±4.81	N/A	9.36±9.55	h–a: 0.65, 0.51
	Cycles/bout	2.2±1.41	N/A	3±5.94	
	Bout duration (min)	0.5±0.5	N/A	0.7±1.3	h–a: 1.2, 0.22
Abdomen rubbing	No. of bouts	4.1±4.3	N/A	N/A	N/A
	Cycles/bout	0.6±0.5	N/A	N/A	
	Bout duration (min)	1.5±1.9	N/A	N/A	N/A
Tarsal tapping	No. of bouts	2.7±2.1	1.5±0.7	N/A	**h–c: −2.15, 0.03**
	Cycles/bout	1.1±0.3	1	N/A	
	Bout duration (min)	1.3±1.8	0.2±0.1	N/A	h–c: −0.45, 0.65
Female tap-groom	No. of bouts	N/A	3.5±3.5	2.2±1.9	c–a: 1.04, 0.29
	Cycles/bout	N/A	5.4±4.4	7.1±8.8	
	Bout duration (min)	N/A	0.4±0.2	0.4±0.2	c–a: −0.15, 0.88
Guarding	Duration (min)	N/A	8. 7±9.1	31.2±55.2	c–a: 0.53, 0.59

We report means and standard deviations. We also report comparisons for each pair of species conducted with Wilcoxon tests. We indicate the species being compared with the first letter of their epithets (e.g. h = *G. howdenae*; c = *G. conwayensis*; a = *Galapaganus ashlocki*). Significant comparisons in bold. N/A indicates comparisons we did not make because the behaviour does not occur in a species (Table 1). With only 4 of 31 tests being significant, Moran's (2003) criterion suggests some risk of spurious significance, but probably not for all cases.

Duration of Behaviours

We did not find significant differences in duration for the courtship behaviours or guarding between the species that performed them (Table A1). However, overall mating duration (between initial mounting and dismounting when there was at least one successful intromission) was significantly longer in the endemics (*G. conwayensis* and *G. ashlocki*) than in the introduced species. Intromissions were significantly longer in the highland endemic (*G. ashlocki*) than in the introduced species (*G. howdenae*), while intromissions in the lowland endemic (*G. conwayensis*) were intermediate (and not significantly different from either of the other species) (Table A1).

We compared the relative duration of different intromissions in the 12 matings where we observed multiple intromissions. In all three species, the relative duration of the second intromission, measured as the fraction of total intromission time, was longer than any other intromission (Table A2: significant intromission order term; nonsignificant species and species∗intromission order interaction terms) (Appendix, Fig. A1). Interestingly, the duration of single intromissions (mean ± SD = 19.3 ± 22.5 min, $N = 40$) was similar to that of second intromissions in multiple-intromission matings (mean ± SD = 23.6 ± 26.9 min, $N = 12$; Wilcoxon test: $Z = 0.3$, $N = 12$, $P = 0.70$).

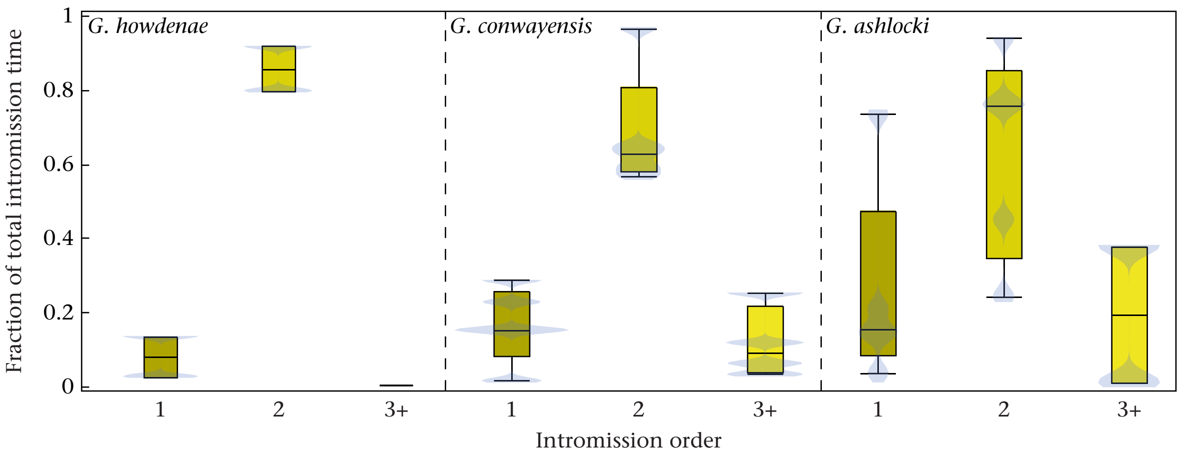

Figure A1. Relative duration of different intromissions in *Galapaganus* species (measured as a fraction of total intromission time in matings with multiple intromissions). Box plots show the median and range. Violin plots show the distribution and density of the data. Note that in the *X* axis we show bins for the first, second and third plus subsequent intromissions (as only a few matings had more than three intromissions; Table A1).

Table A2
Analysis of variation in relative intromission duration according to intromission order and species in the *Galapaganus* weevils

Intromission duration[a]	*df*	*F*	*P*
Intromission order	2, 22	**26.40**	**0.0001**
Species	2, 22	0.13	0.88
Intromission order∗species	4, 22	1.04	0.41

[a] Expressed as fraction of total intromission time. Significant terms in bold.

Transitions between Behaviours

In all species, the main sequence in matings was mounting followed by intromission with genital pumping (Fig. A2). Species with other courtship behaviours (antennae waving in *G. howdenae* and *G. ashlocki*; tarsal tapping in *G. howdenae* and *G. conwayensis*; abdomen rubbing in *G. howdenae*) alternated between them and genital pumping (Fig. A2). In species with female tap-groom (*G. conwayensis* and *G. ashlocki*), that behaviour alternated with male genital pumping and also with antennae waving in *G. ashlocki* (Fig. A2). In *G. conwayensis* and *G. ashlocki*, there were frequent female tap-groom bouts during intervals between intromissions (Fig. A2). In both endemics, withdrawal from the last intromission was followed by guarding and eventual dismount (with guarding more frequent in *G. conwayensis*) (Fig. A2).

There were five (out of 16) *G. ashlocki* matings in which male antennae waving and female tap-groom co-occurred. In those cases, we observed a pattern where bouts or individual cycles of male antennae waving alternated back and forth with bouts or individual cycles of female tap-groom, each behaviour either leading or following on different occasions (Fig. A3). Each mating varied in the number of switches between the two behaviours (Fig. A3: 1–2 switches in (a), 2 switches in (b), 4 switches in (c), 1 switch in (d) and 2 switches in (e)). By contrast, bouts of male genitalic pumping and female tap-groom are an example of what we do not consider alternation.

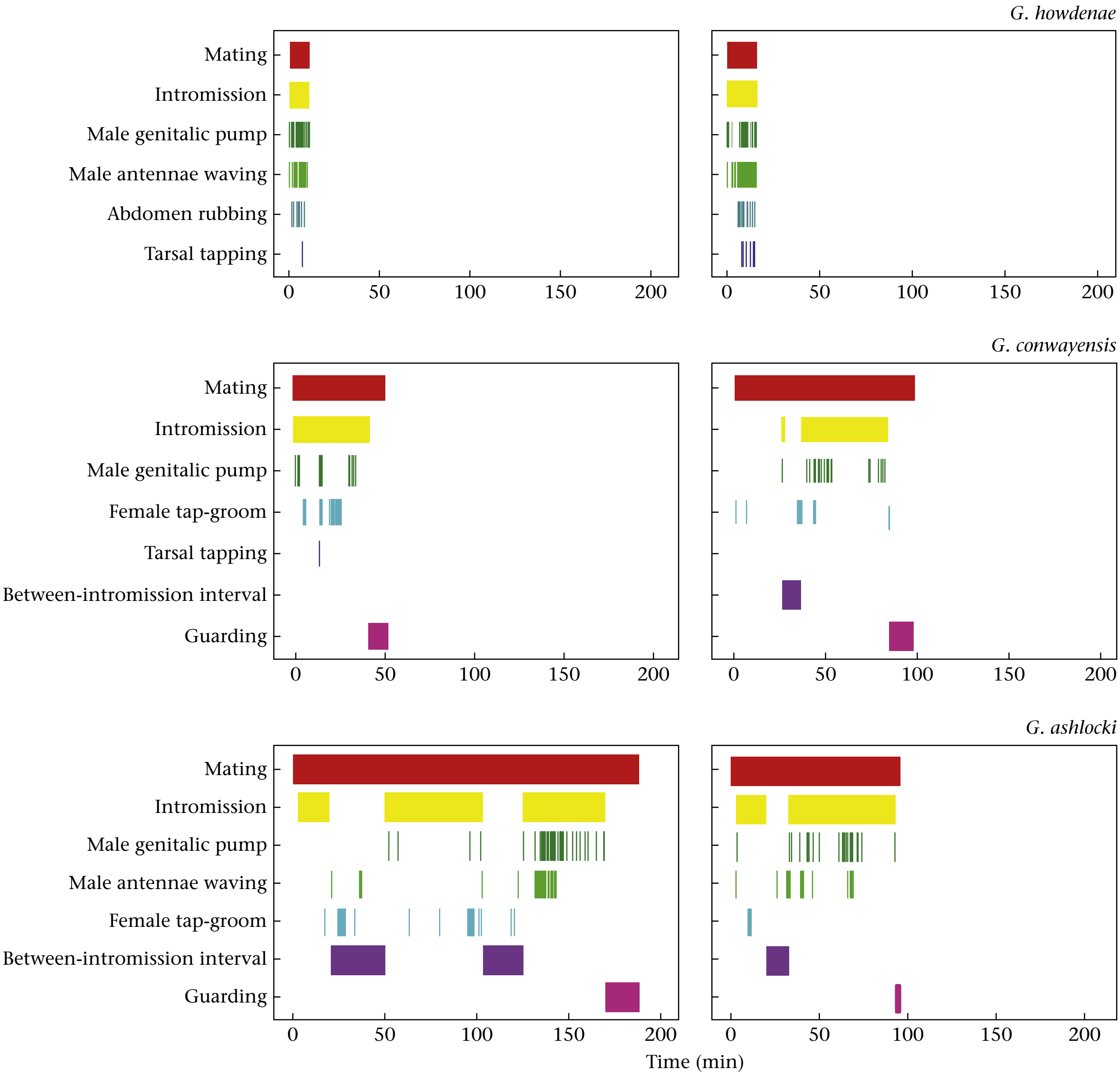

Figure A2. Examples of the course of matings of the three *Galapaganus* species, showing two matings for each species. For each mating, time = 0 is the start of mounting. Individual vertical markers indicate an instance of a behaviour being performed (e.g. one genitalic pump). Behaviours or events that occur along intervals (e.g. intromission) are indicated as continuous.

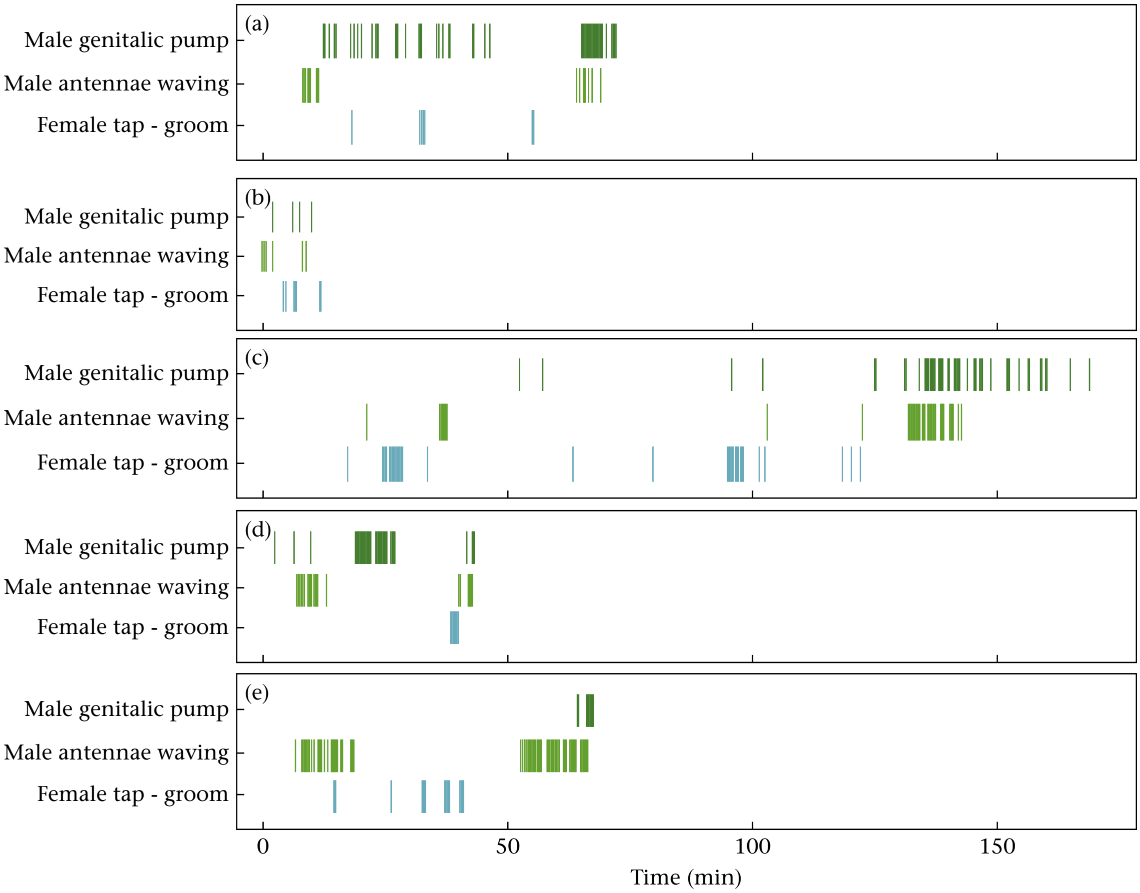

Figure A3. Course of the five (out of 16) *G. ashlocki* matings in which there was coordination or back-and-forth between male and female behaviours. For each mating, time = 0 is the start of mounting. Individual vertical markers indicate an instance of a behaviour being performed (e.g. one genitalic pump, one wave of the antennae). Note that bouts of male antennae movement and female tap-groom occur with some coordination.