



Mating before host colonization: a common trait in outbreeding Scolytinae, potentially linked to invasiveness

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With 3 figures

Abstract: Little is known about the influence of mating strategies that could potentially facilitate the colonization of new hosts in outbreeding species of the weevil subfamily Scolytinae. Individuals typically emerge from their host tree, disperse, and then mate with unrelated conspecifics in a new host where the females establish maternal galleries. Yet, in several species commonly classified as outbreeding, females have been found already mated before host colonization. Precolonization mating provides female with a sperm supply before they find a new host and allows them to establish a maternal gallery on their own. We compared the proportion of females mated before host colonization across 18 European and four American outbreeding Scolytinae species using a phylogenetically controlled analysis. To this end, we determined whether females caught in the spring had sperm in their spermathecae. We found that a proportion of females (range: 16–100%) mated before host colonization in all 22 species. Moreover, this trait was biased, although not significantly, toward invasiveness. Species known to have established outside their native range (Scolytinae with an Invasion History – SIH) displayed a higher proportion of females mated before host colonization than did species restricted to their native range (non-SIH). In *Hylurgus ligniperda* (Fabricius), a Palearctic species currently present across the globe, the proportions of females mated before host colonization reach 90% in the species' native range and up to 99% in its nonnative range (Argentina and New Zealand). Overall, these results show that precolonization mating is widespread among the Scolytinae. This trait could enhance the invasive capacities of outbreeding species by allowing females to establish a maternal gallery independently of any male during colonization, thus facilitating the establishment and spread of species introduced in new geographical areas.

Keywords: Invasive capacity, mating strategy, precolonization mating, pest, invasion

1 Introduction

The wide and diverse Scolytinae subfamily comprises about 6,500 species, mostly living in the phloem and sapwood of a large range of dead or living host plants. Some species are inbreeding, others are outbreeding, and a minority reproduce through parthenogenesis (Kirkendall 1983).

In inbreeding Scolytinae, females mate with a relative in their natal host, then emerge, disperse, and find a new host in which they establish a maternal gallery on their own (Kirkendall 1983). Theoretically, it would take just one mated female to establish a population in a new geographical area. Thus, the invasiveness of scolytine species might be enhanced via inbreeding (Jordal et al. 2001; Lantschner et al. 2020; Grégoire et al. 2023). In contrast, in outbreeding species, individuals typically emerge from their natal host, disperse, and then mate with unrelated conspecifics in a new host where the females establish maternal galleries. In monogynous species, females are generally the pioneer sex. They initiate gallery construction in their new host following emergence and are later joined by a single male. In contrast, in polygynous species, males are the pioneer sex and are joined by several females (Kirkendall 1983).

To date, outbreeding Scolytinae have been treated as a homogeneous category. Yet, in certain outbreeding species, a proportion of females have already mated before their colonization of breeding material (i.e., precolonization mating). This situation can result from three different processes: pre-emergence mating, postdispersal mating or re-emergence (Fig. 1; Kirkendall 1983; Kirkendall et al. 2015). Pre-emergence mating (in the natal host) and postdispersal mating (during maturation feeding or overwintering in a new host) are defined as a first mating between related (=inbreeding) or unrelated individuals (=outbreeding) before colonizing a new host tree to establish a first maternal gallery (e.g., *Orthotomicus erosus* Wollaston, *Hylurgus ligniperda* (Fabricius), *Ips grandicollis* (Eichhoff), *Scolytus multistriatus* (Marshall), *Tomicus piniperda* L., *I. pini* Say, *Dendroctonus ponderosae* Hopkins, *I. typographus* (L.) (Dacquin et al. 2024 and references therein)). Moreover, females can be mated before infesting a new host because

they re-emerge from a previous gallery, after oviposition (Kirkendall 1983). These reproductive strategies increase mating opportunities for outbreeding species and allow females to establish a gallery independently from any male (All & Anderson 1972). The ability of females to create galleries on their own could be a considerable advantage in outbreeding species, especially when the density of individuals is low and/or if individuals disperse far away from their source populations, which reduces their likelihood of encountering a mate, an important component of Allee effects. In these cases, a solitary founder could lower Allee thresholds by removing the need to find a sexual partner during host colonization (Shaw & Kokko 2014 and references therein).

The Scolytinae include some of the world's most formidable forest pests (Kirkendall et al. 2015). During its recent bark beetle crisis (2018–2021), Europe lost millions of m³ of spruce, mainly due to the eight-toothed spruce bark beetle, *I. typographus* (L.). This was favored by exceptional hot and dry seasons, and particularly affected even-aged, monospecific stands (Hlásny et al. 2021; Saintonge et al. 2022). In addition to the damage they can cause to trees in their native range, many species have become established in other parts of the world, where they can pose a serious risk to plant health (Haack & Rabaglia 2013; Grégoire et al. 2023). Among these, the ambrosia beetle, *Xyleborus glabratus* Eichhoff, is arguably one of the most damaging Scolytinae in its introduced range. Native to Asia, this beetle and its symbiotic pathogenic fungus, *Raffaelea lauricola* T. C. Harr., Fraedrich & Aghayeva, were accidentally introduced into the USA, where their association caused laurel wilt disease that has destroyed more than 500 million trees since the early 2000's (Hughes et al. 2017). In its native range, this species has relatively insignificant effects on forests; it primarily targets weakened or dead trees. However, in its nonnative range, together with *R. lauricola*, it also infests and kills healthy trees and constitutes a significant threat to tree health (Huler & Dunn 2011).

Scolytinae known to have established outside their native range (henceforth called Scolytinae with an Invasion History or SIH; Grégoire et al. 2023; EFSA et al. 2024) typically share several traits, including sibling mating, polyphagy, host

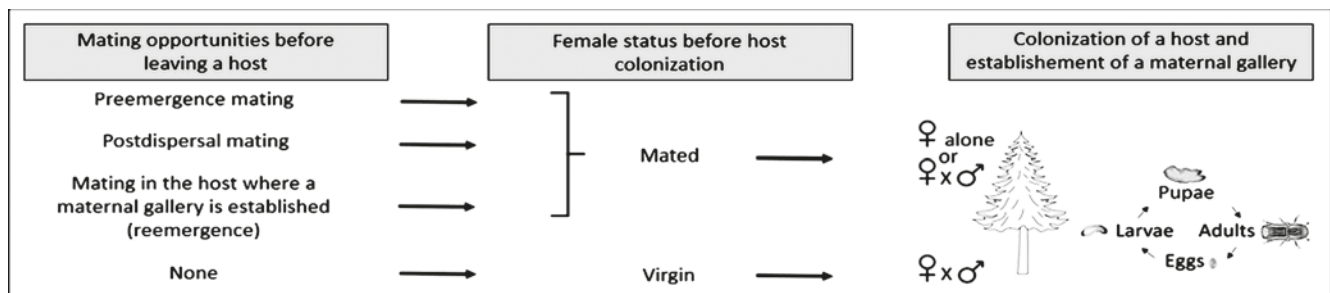


Fig. 1. Mating opportunities and female status before host colonization in Scolytinae.

plant abundance in the invaded area, symbiotic associations with fungal pathogens, and associations with wood commodities (Jordal et al. 2001; Lantschner et al. 2020; Grégoire et al. 2023). Among these characteristics, mating strategies seem to play a decisive role. Grégoire et al. (2023) reported that 53.4% (87 out of 163) of SIH species are inbreeding, a proportion significantly higher than the 27% of all scolytines classified as such (Kirkendall et al. 2015).

In this study, we used phylogenetically controlled analyses to compare the proportion of females mated before host colonization in the spring across 18 European and four American outbreeding scolytine species. First, we tested whether the percentages of females that had mated before host colonization were higher for SIH than non-SIH species. Second, we compared the proportions of females mated before host colonization between monogynous versus polygynous species. We predicted that precolonization mating should be favored when gallery foundation is initiated by females, and levels should therefore be higher in monogynous than polygynous species. Finally, we explored whether, for a given species, precolonization mating might be more common in the non-native versus native ranges. To this end, we compared the proportion of females mated before host colonization in *H. ligniperda* between the species' native range (Europe) and nonnative range (Argentina and New Zealand).

2 Materials and methods

2.1 Sampling in Europe

In spring 2022, 18 scolytine species were trapped or collected as they emerged from infested logs, after the overwintering period. Sampling took place at 15 locations across 9 European countries (Belgium, Czechia, France, Hungary, Italy, Poland, Portugal, Slovakia, and the United Kingdom; Supplementary Fig. S1). Individuals were collected at several locations to increase the chances of collecting as many target species as possible. We also wished to compare the proportion of females mated before host colonization within species among locations. The range of species captured in Europe was largely dependent on the local populations. Based on the literature and our own knowledge of the field, we had a rough idea of the genera that could be captured; however, we were unable to predict the exact species composition until the traps were checked. The traps were baited with α -pinene (0.3 g/day at 20 °C), ethanol (2 g/day at 20 °C), and a mixture of Ipsenol ((4*S*)-2-methyl-6-methylideneoct-7-en-4-ol) and Ipsdienol ((4*S*)-2-methyl-6-methylideneocta-2,7-dien-4-ol) at a release rate of 2 mg/day at 20 °C (Econex, Spain). In Belgium and Hungary, lineatin (3,3,7-trimethyl-2,9-dioxatricyclo[3.3.1.0]nonane) (Econex, Spain) was also added to some traps to attract *Trypodendron* species. The traps were deployed in beech, pine, or spruce stands. The beetles were captured using traps containing a solution of 70% propylene glycol. The traps' contents were collected

approximately every two weeks and transferred to vials containing 70% ethanol. The period during which the sampling was conducted varied between locations, typically lasting around 1 to 3 months. The beetles were collected early during the spring to avoid collecting too many re-emergent females and have a stronger focus on females mated before emergence or during postdispersal phases.

2.2 Sampling in Argentina and New-Zealand

To assess the proportion of *H. ligniperda* females that had mated prior to host colonization in localities outside its native range, an additional sampling was conducted during spring 2022 in Argentina and New-Zealand. The trapping methodology was identical to that established in Europe except that traps were exclusively baited with α -pinene and ethanol, and deployed in pine stands.

2.3 Sampling in USA

2.3.1 Mississippi – *D. frontalis* Zimmermann

The Mississippi southern pine beetle spring trapping campaign was conducted in 11 counties. In each, three 12-unit Lindgren Funnel traps were deployed between mid-March and early April 2023. Each trap was placed in a small stand of deciduous trees separated by at least 50 m from the nearest pine tree to minimize chances of initiating bark beetle infestations on surrounding pine trees. The deciduous stands where traps were placed were surrounded by pine forests containing primarily a loblolly pine (*Pinus taeda* L.) overstory. Traps were hung from branches ~ 1.5 m above the forest floor and baited with α -pinene (2 g/day at 25 °C) and a frontalin lure (5 mg/day at 25 °C) hung directly from the trap, and an endo-brevicomin lure (~0.3 mg/day at 25 °C) hung 5 m adjacent to the trap (Sullivan and Mori 2009). Trap cups were filled with a 50:50 mixture of propylene glycol and water and checked weekly for four weeks. Five out of the 11 counties (Supplementary Fig. S2) were chosen for the analyses of the proportions of females mated before host colonization, based on a sufficient number of trapped beetles ($n > 20$).

2.3.2 Georgia – *I. avulsus* (Eichhoff), *I. calligraphus* (Germar) and *I. grandicollis* (Eichhoff)

Ips bark beetles were sampled in Georgia using Lindgren Funnel traps distributed in six plots with two traps at each plot in *P. taeda* dominated stands. Each plot had one trap baited with *Ips* pheromones (-)-ipsenol (0.6–0.8 mg/day at 25 °C), (+/-) ipsdienol (0.6–0.8 mg/day at 25 °C), and *cis*-verbenol (0.55 mg/day at 25 °C) and one trap baited with ultra-high-release ethanol (200–400 mg/day at 25 °C) and α -pinene lures (2 g/day at 25 °C). Traps were placed 15 m apart, and plots were placed 100 m apart. Traps were installed on 7 March 2023, and removed on 11 April 2023, with collections proceeding every week for a total of four

collections. Each collection cup was filled with a 50:50 mixture of propylene glycol and water to preserve samples in the field. Once collected, the insects were stored in a lab freezer (-18°C) until sorting and identification. *Ips* beetles were identified and placed into separate vials with 95% EtOH.

2.4 Scolytinae with an invasion history (SIH) vs. Scolytinae with no invasion history (non-SIH)

All European individuals were identified using the taxonomic key developed by Balachowsky (1949). In our analyses, we only retained species known to outbreed (Kirkendall et al. 2015). They were classified into two categories: Scolytinae known to have established outside their native range (i.e., SIH) or restricted to their native range (i.e., non-SIH), based on Munro (1926), Mandelshtam et al. (2006), Faccoli et al. (2020), Lantschner et al. (2020), Bright (2021), and Grégoire et al. (2023). We opted not to use the term “non-native Scolytinae” in place of “Scolytinae with an invasion history” because all the species were captured within their native range, except *I. cembrae* (Heer) which was sampled in Ludlow (UK) and *H. ligniperda* which was sampled in both its native and nonnative ranges. Our definition of SIH does not necessarily imply a worldwide distribution but it aligns with the concept of species establishing outside their native range through human-mediated transport without necessarily being invasive.

2.5 Female status before host colonization

We determined the mating status of females according to Dacquin et al. (2024). Briefly, females were dissected under a stereo microscope (Leica MZ6; 100x magnification), and the presence/absence of sperm in the spermathecal sac and pump was visually determined (see Dacquin et al. 2024). The dissected individuals were randomly taken from the vials. When possible, a minimum of 30 females were dissected per sampling date and per location.

For each species, we calculated the overall percentage of females mated before host colonization. First, we summed the number of mated females for each collection period within the different sampling locations (see exceptions in Supplementary Tables S1 and S2). This sum was then divided by the total number of mated and virgin females for the corresponding period and location. The number of individuals sampled per species and location varied greatly. Indeed, in some species of bark beetle, population densities can be naturally low and fluctuate seasonally. It is for this reason that we have pooled the number of insects analyzed across different dates and locations to obtain an overall percentage for each species.

2.6 Phylogenetic analyses

We applied a phylogenetic approach because the study species are not evolutionarily independent from each other.

Searching the literature, we found no preexisting phylogeny that comprises all the species sampled in this study. Therefore, we built our own using genetic sequencing data for a mitochondrial gene (cytochrome c oxidase subunit 1 [CO1]) that is in the NCBI repository (sequence accession numbers: Supplementary Table S3). The CO1 gene is the only gene for which sequences were consistently available across all the species included in the present study. We aligned the sequences using the Muscle algorithm in MEGA v. 11.0. Sequences were then cut manually to limit the amount of missing data (see the FASTA file in Supplementary).

We reconstructed the species' phylogeny using Bayesian approaches. The Bayesian method was implemented in BEAST v. 1.10.4. We used the HKY+I+G model of sequence evolution (selected via *ModelFinder Plus*), a strict molecular clock model, and a Yule model for the tree prior. Moreover, we constrained our analysis to align with Jordal & Cognato (2012), Cognato (2013) and Pistone et al. (2018), whose phylogenies are based on multiple genes and include a large number of Scolytinae species (e.g., *Dryocoetini* and *Ipini* as sister tribes). We performed two independent Markov chain Monte Carlo (MCMC) runs, each comprising 20 million generations; sampling occurred every 100,000 iterations. The first 10% of trees sampled from the posterior distribution were discarded as burn-in. The convergence and mixing properties of the MCMC chains were assessed using LogCombiner v. 1.10.4 and Tracer v. 1.7.2, ensuring that the effective sample size values for the estimated parameters exceeded 200. Finally, from the posterior distribution of trees, we used TreeAnnotator v. 1.10.4 to extract a maximum clade credibility (MCC) tree, which served as the final maximum-likelihood phylogeny. The MCC tree was edited for clarity employing FigTree v. 1.4.4.

The tree was rooted using *Cossonus linearis* (Fabricius) (Curculionidae; Cossoninae), and *Pissodes yunnanensis* Langor and *Pimelocerus perforatus* (Roelofs) (Curculionidae; Molytinae) as outgroup species which were subsequently pruned. The resulting tree's original branch lengths were adjusted to create an ultrametric tree, which reflected relative time spans. We performed this adjustment using a semiparametric smoothing method based on penalized likelihood that was implemented using the R package *ape*. We assigned a conservative value of 0.1 to the smoothing parameter.

2.7 Statistical analyses

To assess the strength of the phylogenetic signal associated with the overall percentages of females mated before host colonization, we calculated Pagel's λ using the function *phylosig* in the R package *phytools*. This metric reflects the level of association between species traits and phylogenetic history. Values closer to 0 indicate that traits have evolved independently of species' phylogenetic relationships, while values closer to 1 indicate traits are fully aligned with species' phylogenetic relationships (Lemey et al. 2010).

For our comparisons, we first focused on scolytines caught within their native ranges in Europe and USA because nonnative species can evolve distinct mating strategies in their nonnative ranges, as documented in other species than Scolytinae (Wong & Candolin 2015 and references therein). We used phylogenetically controlled generalized least-squared (PGLS) regressions (*ppls* function in the R package *caper*) to compare the overall percentages of females mated before host colonization for SIH *versus* non-SIH and for monogynous *versus* polygynous Scolytinae species. Moreover, a PGLS analysis was performed to assess the relationship between the overall percentage of females mated before host colonization and the number of land-masses where the species is established (see Supplementary Table S4).

The above analyzes did not include *H. ligniperda* from outside their native European range. We used a Fisher's exact test in a separate analysis to compare the proportion of *H. ligniperda* females mated before host colonization in their nonnative range (sampled from Argentina and New Zealand) and in their native range (from Czechia, France and Portugal).

3 Results

3.1 Precolonization mating

The sampling locations and periods, the number of females dissected, and the percentages of females mated before host colonization are given in Supplementary Tables S1 and S2. All 22 study species included females mated before host colonization. The overall percentages of females mated before host colonization ranged from 16% to 100% across species (Fig. 2). The percentages of females mated before colonization varied significantly among locations for *D. frontalis*, *Hylurgops palliatus* (Gyllenhal), *I. sexdentatus* (Boerner), *O. erosus*, *O. laricis* (Fabricius), *Trypodendron lineatum* (Olivier), and *T. domesticum* (L.). Moreover, in some species (e.g., *I. typographus* from Beauraing (BE) and Jelesnia (PL), and *I. sexdentatus* from Stará Boleslav (CZ)), the percentage of females mated before host colonization increased over the sampling period, possibly due to female re-emergence (Supplementary Table S1).

The Bayesian analyses resulted in a phylogenetic tree highly concordant with previous Scolytinae phylogenies (Jordal & Cognato 2012; Cognato 2013; Kirkendall et al.

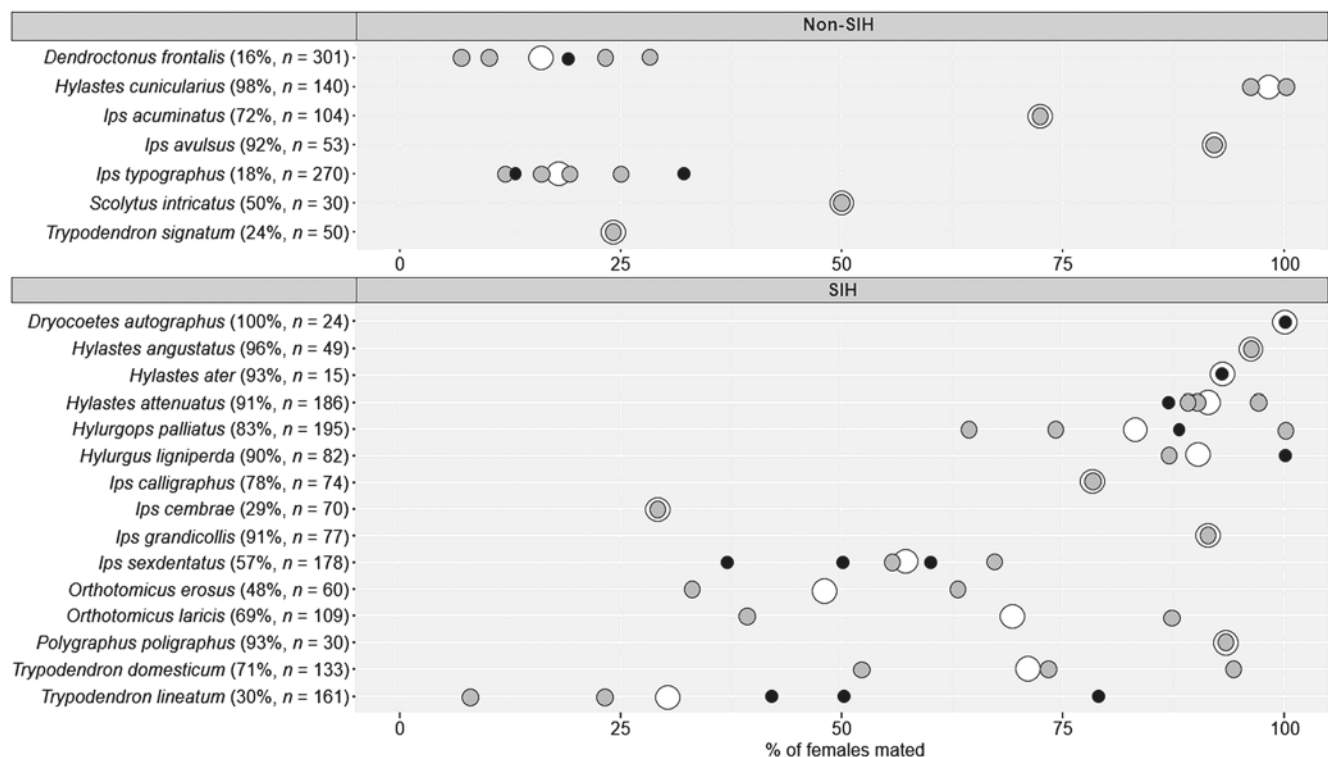


Fig. 2. Percentages of females mated before host colonization in 22 Scolytinae species in Europe and USA. The empty circles represent the species-specific overall percentages of females with sperm in their spermathecae before host colonization. The filled circles are the species-specific percentages of females with sperm in their spermathecae before host colonization across the different sampling locations. In some cases, data were combined across different sampling periods for a same sampling location (see Supplementary Tables S1 and S2). Small black filled circles: $n < 30$; larger grey filled circles: $n \geq 30$. The numbers in the parentheses next to the species names are the overall percentages of females mated before host colonization and the total number of females dissected.

2015; Pistone et al. 2018; Guo et al. 2023). Closely related species did not display a high degree of trait similarity: Pagel's λ for the overall percentage of females mated before host colonization was equal to 0.56 and did not significantly differ from 0 ($p = 0.1$).

3.2 Scolytinae with an invasion history (SIH) vs. Scolytinae with no invasion history (non-SIH)

Among the SIH, nine out of the 15 species displayed a high level of females mated before host colonization (>75%), against just two out of the seven non-SIH species (Fig. 2). The proportion of females mated before host colonization was higher, though non-significantly, in SIH (mean $\pm$ SD: 74.6 ± 23.5) than non-SIH (53 ± 35.2) when accounting for phylogeny (PGLS regression: $t = 1.6$, slope $\pm$ SE = 19.86 ± 11.95 ; $\lambda_{\text{residuals}} = 0.3$, $p = 0.11$) (Fig. 3).

3.3 Landmasses

There was no relationship between the overall percentage of females mated before host colonization and the number of landmasses where the species is established (PGLS regression: $t = 1.2$, slope $\pm$ SE = 1.91 ± 1.59 ; $\lambda_{\text{residuals}} = 0.5$, $p = 0.24$) (Supplementary Table S4).

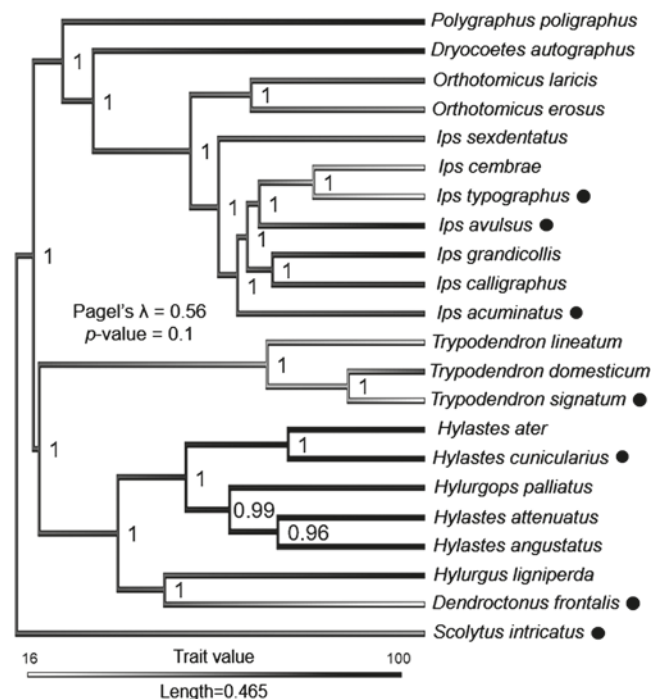


Fig. 3. Phylogenetic tree for the 22 Scolytinae species. The posterior probability values are indicated to the right of each node. The branches are shaded (white to black) according to the values of the overall percentages of females mated before host colonization. The lowest values are in white, and the highest values are in black. The filled circles next to the species names indicate non-SIH species.

3.4 Polygynous vs. monogynous species

There was no difference in the proportions of females mated before host colonization between polygynous and monogynous Scolytinae species when accounting for phylogeny (pgls regression: $t = -0.26$, slope $\pm$ SE = -3.47 ± 12.99 ; $\lambda_{\text{residuals}} = 0$, $p = 0.79$). The mean overall percentages of females mated before host colonization did not differ between monogynous (mean $\pm$ SD: 70.1 ± 31.5) and polygynous species (64.7 ± 26.4).

3.5 Native vs. nonnative range in *H. ligniperda*

The overall percentages of *H. ligniperda* females mated before host colonization differed significantly between the species' native range (Europe: 90%, $n = 82$) and nonnative range (Argentina and New Zealand: 99%, $n = 115$) (Fisher's exact test: $p = 0.004$; Supplementary Table S1).

4 Discussion

Our results provide two important insights into the mating strategies and invasiveness of outbreeding Scolytinae. First, we found that females mated before host colonization in spring were present in all 22 study species. Overall percentage values ranged from 16% to 100%. Second, the proportion of females mated before host colonization was higher in SIH than non-SIH species. These findings demonstrate that mating before host colonization is common among outbreeding Scolytinae and suggest they may play a role in their successful establishment in new geographical regions. The significantly higher percentage of females mated before host colonization in the nonnative range as compared to the native range in *H. ligniperda*, a Palearctic species currently present across the globe, further supports the assumption that precolonization mating could facilitate the establishment of Scolytinae in new geographical areas.

4.1 Invasive capacities and mating strategies

Our comparative analysis reveals that, among the 22 study species, the proportion of females mated before host colonization was higher in SIH than non-SIH species. We therefore hypothesize that outbreeding species engaging in a substantial degree of precolonization mating should be well represented among SIH species. This trait did not exhibit a phylogenetic signal, which might otherwise have explained why tribes or genera with this trait are overrepresented among SIH species (Grégoire et al. 2023). A high proportion of females mated before host colonization could be advantageous when species are introduced in new geographical areas, where population densities are initially low, providing them with similar benefits as inbreeding Scolytinae, namely, they could bypass the need to find a mate during the colonization of a host. However, inbreeding depression could influence the reproductive success of solitary females (Dacquin et al. 2024 and references therein). Furthermore, even though our sampling

covered a large range of species, we recognize that the lower representation of non-SIH species in the sample could have biased our results. Further studies encompassing a broader range of species are needed to consolidate our findings and offer a more comprehensive understanding of the observed pattern. It would also be highly valuable to investigate the role of precolonization mating on invasiveness across multiple species within the same genus. For example, eight species within the genus *Scolytus* have documented invasion histories (Grégoire et al. 2023). Among these, *S. multistriatus*, *S. schevyrewi* A.P.Semenov Tjan-Shansky, and *S. sulcifrons* Rey are known vectors of Dutch Elm Disease, *Ophiostoma ulmi* (Buisman) Nannf. and *O. novo-ulmi* Brasier, a trait that may have facilitated their establishment in new territories by reducing the propagule pressure needed to colonize new hosts. Comparing species within the *Scolytus* genus that engage in precolonization mating with those that do not, and those that vector plant pathogens with those that do not, would provide valuable insights.

We found that monogynous and polygynous species displayed equivalent proportions of females mated before host colonization. In monogynous species, females are generally the pioneering sex; in contrast, in polygynous species, males initiate the infestation on the new host. Vilardo et al. (2022) found that, among European pine bark beetles known to have established in new geographical areas, monogynous species are more common than polygynous species. Although there was no difference in the percentages of females mated before host colonization between the two species types, precolonization mating might particularly help monogynous species to establish in new geographical areas by providing greater postemergence independence to single females.

4.2 Native vs. nonnative range in *H. ligniperda*

In *H. ligniperda*, the proportion of females mated before host colonization was 90% in the species' native range and reached up to 99% in its nonnative range. While this result is consistent with precolonization mating being associated with invasiveness, additional studies across multiple genera are required to draw broader conclusions regarding variations in mating behaviors between native and non-native ranges within the Scolytinae. Past reviews have explored how genetic bottlenecks and selective pressure in new environments could have brought changes in behavioral traits affecting a species' capacity to establish and spread in new geographical areas (Wong & Candolin 2015). A well-known example is the case of the Argentine ant (*Linepithema humile* (Mayr)) which develops highly unicolonial populations in its nonnative range (e.g., Chile and California) but to a lesser extent in its native range. This shift involves a reduction in levels of intraspecific aggression, which subsequently allows a notable increase in population densities (Suarez et al. 1999).

4.3 Knowledge gaps

More than 4,000 Scolytinae species are currently classified as outbreeding (Kirkendall et al. 2015). Remarkably, we found that all our study species displayed a proportion of females mated before host colonization in the spring. However, the finer biological details related to this phenomenon remain unknown given that scolytines live cryptic lives under the bark of trees. Little information is available about kinship among sexual partners or the actual frequency of pre-emergence and postdispersal mating in the field. Moreover, re-emerging females (i.e., which had already laid eggs before overwintering or in early spring before being caught in the traps) may constitute a part of the mated females recorded in the various studies which investigate pre-emergence and postdispersal mating, including the present work (Dacquin et al. 2024 and references therein). Data on kinship and female emergence *versus* re-emergence are lacking in our study because of (i) the uncertainty around the identity of the females' mates (i.e., relatives *vs.* nonrelatives) and (ii) the challenge of distinguishing first-time, early mated females from re-emerging females. The presence or absence of *corpora lutea* in freshly sampled or well-preserved individuals (e.g., in Bouin's solution) could help distinguish between the two groups. However, this was not made possible in this study because our specimens were placed in propylene glycol upon capture and then preserved in ethanol for several weeks/months.

We also observed that the percentages of females mated before host colonization differed between geographical locations for several species (e.g., *D. frontalis*, *O. laricis*, *T. domesticum*, *T. lineatum*; Supplementary Table S5). Both biotic (e.g., host availability, predator pressure) and abiotic variables (e.g., temperature, elevation, rainfall) differed among these locations and could directly impact local scolytine populations, affecting their development time, density and proximity under the bark, or overwintering survival. Ultimately, environmental conditions could also influence the likelihood of pre-emergence mating, postdispersal mating and/or the number of parent females that re-emerge in spring. Intraspecific differences in the prevalence of precolonization mating could also have a genetic basis. These complex issues merit further research.

4.4 Gallery establishment by unpaired females

Our results suggest that, although little studied, precolonization mating is probably quite common in outbreeding species, given the high number of females mated before host colonization found in most species. Understanding the mating strategies of Scolytinae species provides valuable insights into their reproductive behaviors and population dynamics, which are both important contributors to invasiveness. By engaging in precolonization mating, females can emerge with a supply of sperm and thereby establish mater-

nal galleries on their own. This scenario is clearly illustrated by *I. grandicollis* (Eichhoff) in Australia, a polygynous species whose females present a high proportion of females mated before host colonization (> 80%; Witanachchi 1980), who are known to engage in pre-emergence mating, and can establish galleries on their own (All & Anderson 1972).

Despite these findings, research in this area has generally failed to consider the possibility of female precolonization mating and the establishment of system galleries by unpaired females. For instance, Köbayashi & Takagi (2020) observed that 70.4% of the galleries occupied by *Polygraphus proximus* Blandford contained females, eggs, and larvae, but no male. They suggested that the explanation was re-emergence dynamics, with males re-emerging before females. In a study on *Phloeosinus armatus* Reitter, Baruch et al. (2017) noted that 21.5% of 172 galleries contained eggs and a solitary female. Reid (1999) characterized the presence of adult *I. latidens* (LeConte) in 131 galleries, aged 1 to 40 days, and found that 35.8% contained a solitary female. In these examples, the absence of males could be explained by re-emergence, but it could also be explained by female precolonization mating and gallery started and established by unpaired females.

However, we can expect that, when population density is high, even if females mate prior to host colonization, they may join or be joined by a male, or, in polygynous species, rely on male signals to reach a suitable host. For instance, within their native range, *I. typographus* females are unlikely to face a short supply of males given pheromone-mediated collective foraging (Vité et al. 1972), balanced sex ratios at emergence (Wermelinger 2004), unbalanced sex ratios among mates in newly established galleries (one male for one to four females; Wermelinger 2004), and they generally do not excavate the bark by themselves (Paynter et al. 1990).

4.5 Conclusion

A better understanding of a pest's biology improves our capacity to identify the ecological and economic risks it represents, prevent new invasions, or mitigate the effects of established populations. In the weevil subfamily Scolytinae, previous research has shown a disharmony among SIH species, with inbreeding species being overrepresented. The present study shows that outbreeding species may engage in a substantial degree of precolonization mating and that this trait could be potentially associated with invasiveness. Together, these findings suggest that precolonization mating could be used as an additional criterion to pinpoint species with a higher risk of establishment outside of their present range, providing increased support for pest risk assessments and perhaps allowing for better quarantine procedures and control methods. Finally, we encourage further studies to explore precolonization mating in greater depth to better understand this reproductive strategy and its potential influence on invasive capacity.

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Supplementary Fig. S1, S2; Table S1–S5