



Natural history and behaviour of *Dunatothrips aneurae* Mound (Thysanoptera: Phlaeothripidae), a phyllode-gluing thrips with facultative pleometrosis

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Thrips of the genus *Dunatothrips* (Thysanoptera: Phlaeothripidae) construct domiciles by tying phyllodes of Australian *Acacia* trees together with silk-like glue. Females often co-found domiciles (pleometrosis), an apparently cooperative behaviour that potentially provides insights into social evolution. However, little is known about their basic natural history, limiting the scope for testable predictions. Here, we address this crucial prerequisite step by investigating some key outstanding questions in the most common species, *D. aneurae*, on its host, *Acacia aneura*. We detail distribution in space and time, mating, dispersal, domicile building and defence. *Dunatothrips aneurae* was distributed in loosely reproductively synchronized patches, and tended to prefer east-facing, terminal phyllodes on thin-phyllode *A. aneura* varieties. Mature domiciles contained middens, concentrated areas of waste, suggesting active maintenance of domiciles and the potential for the division of labour. We observed inbreeding and outbreeding. *Dunatothrips aneurae* males engaged in short, truncated matings with sisters before dispersing locally, mating with females in nearby immature domiciles; longer distance dispersal, although it must happen, is still undocumented. Males and females mated multiply. Lone females required male presence to initiate domiciles, constructed them without male help and lost wings on nesting by abscission. Silk production occurred well before egg laying. Aggression or defence appeared to be entirely absent. Taken together, these observations suggest that research into co-founding behaviour should focus on: (1) local crowding; (2) lack of aggression; and (3) potential division of labour with respect to egg production, silk production and domicile maintenance. These results should provide a springboard for questions on the potential evolution of cooperation in this species. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, ●●, ●●–●●.

ADDITIONAL KEYWORDS: cooperation – dispersal – joint nesting – nutritional ecology – sexual selection – sociality.

INTRODUCTION

In social insect research, species with facultative joint nest founding ('pleometrosis') are of interest because they can help to elucidate the costs and benefits of breeding singly versus breeding in groups. Pleometrosis plays an important role in the evolution of eusociality via a 'semisocial' route (reproductive castes evolving within aggregations of individuals from the same generation, as opposed to a 'subsocial' route where offspring remain in the natal nest and

help their mother produce more siblings; Wheeler, 1923; Lin & Michener, 1972; Itô, 1993). Traditionally, research has focused on the Hymenoptera (e.g. Rissing & Pollock, 1987; Tschinkel, 1998), but pleometrosis happens in diverse taxa, e.g. termites (Shellman-Reeve, 1997; Hacker *et al.*, 2005), mites (Saito, 1997), aphids (Miller, 1998a, b) and *Acacia* thrips (Morris, Schwarz & Crespi, 2002). Species-specific ecological factors often determine key costs and benefits of pleometrosis, highlighting the importance of species natural history, biogeography and ecology in understanding this behaviour (Richards & Packer, 1998; Tschinkel, 1998; Helms Cahan, 2001; Helms Cahan & Helms, 2012).

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Australian *Acacia* thrips (Thysanoptera: Phlaeothripidae) contain separate origins of (1) eusociality without pleometrosis and (2) pleometrosis without eusociality (Crespi, Morris & Mound, 2004). They provide a particularly insightful comparison with the Hymenoptera, because the two groups share the trait of haplodiploid sex determination (i.e. males and females develop from unfertilized and fertilized eggs, respectively), whose importance in predisposing lineages towards sociality has long been debated (summarized in Herbers, 2009). Eusocial members of the *Acacia* thrips all belong to one genus, *Kladothrips*, and induce galls (hollow fleshy outgrowths of plants with a nutritive inner surface on which the insects feed) on various species of *Acacia*. Eusociality in *Kladothrips* is thought to have evolved via the subsocial route, and has received considerable recent attention (e.g. Crespi, 1992; Kranz *et al.*, 2001; see Crespi *et al.*, 2004 for a review).

Pleometrosis occurs in a separate and much less well-studied clade containing, among others, all known ‘domicile-constructing’ thrips (*Dunatothrips*, *Lichanothrips*, *Paracholeothrips*, *Carcinothrips*, *Sartrithrips*, *Truncatothrips* and *Panoplothrips*). These species live and breed entirely within domiciles made from a silk-like cement (henceforth ‘silk’) extruded from the abdomen, which is used to tie *Acacia* phyllodes together according to a range of methods (Crespi *et al.*, 2004). Pleometrosis appears to be restricted to *Dunatothrips* spp. (Crespi *et al.*, 2004). Some *Dunatothrips* construct ‘tents’ by stretching silk across the surfaces of single phyllodes (e.g. *D. aulidis* Mound & Morris, *D. skene* Mound & Morris; Mound & Morris, 2001), whereas others secure two phyllodes together using an ‘O’-shaped silk wall (*D. armatus* Moulton, J. D. J. Gilbert, pers. observ.); others, such as *D. aneurae* Mound and *D. vestitor* Mound & Morris, tie multiple phyllodes together loosely (Crespi & Mound, 1997).

Existing ecological studies of *Dunatothrips* (Morris *et al.*, 2002; Bono & Crespi, 2006, 2008; Bono, 2007) have focused mostly on the costs and benefits of pleometrosis in the most common member, *D. aneurae*, which lives exclusively on *Acacia aneura* Benth. However, all current knowledge of *D. aneurae*’s biology has been inferred from individuals found inside domiciles instantly frozen or preserved in fluid, such that no behavioural data are available and much of the natural history of the species remains enigmatic. Aside from being interesting in its own right, this natural history could provide critical insight into the evolution of joint nesting in this species, and must be addressed before we can generate detailed, testable hypotheses. For example, basic questions remain, such as:

1. What is the distribution of domiciles among and within trees, in space and time?
2. When, where and how does mating occur, what are its implications for the mating system, and does the species inbreed?
3. How do individuals disperse and form new domiciles?
4. How do inhabitants defend against attack by enemies or conspecifics?

In this article, we focus on these four questions, laying the foundations for deeper investigation of the biology of this species.

METHODS

Dunatothrips aneurae domiciles are often co-founded by several females (Morris *et al.*, 2002), probably co-dispersing sisters (Bono & Crespi, 2008). Co-founding is associated with reduced average per capita reproduction (Bono & Crespi, 2006, 2008) and increased survival (Bono & Crespi, 2006). Foundresses lose their wings (dealate) following nesting, and produce a single generation of offspring that develops entirely within the domicile (Morris *et al.*, 2002). Most adult offspring disperse, but a few may remain and become dealate within, or in an extension of, the natal domicile, given that mature domiciles contain more than the predicted numbers of dealate individuals (Bono & Crespi, 2008). In the field, domiciles are typically found containing original foundresses plus offspring at all stages of development, and only rarely with founding males (Bono & Crespi, 2006). At the landscape level, they reportedly occur patchily and appear to prefer narrow-phyllode varieties of *A. aneura* and, within a tree, terminal phyllodes (Morris *et al.*, 2002).

DISTRIBUTION OF DOMICILES IN SPACE AND TIME

We collected active *D. aneurae* domiciles from *A. aneura* populations at the University of New South Wales Arid Zone Research Station, Fowlers Gap, NSW 2880, Australia (‘Fowlers Gap’), approximately 110 km north of Broken Hill (see Table 1 for GPS locations) during four short field trips in October 2011, March 2012, August 2012 and October 2012. Trees were initially searched from several angles using close-focus binoculars; if domiciles were discovered, the tree was exhaustively searched. The number of domiciles present on each inhabited branch was noted. The compass bearing of each inhabited branch was taken facing outwards along the line joining the trunk to the rough centroid of the phyllodes on the branch. In a subset of inhabited trees, the entire branch architecture of the tree was recorded. We

Table 1. Sites from which *Dunatothrips aneurae* domiciles were collected. All sites refer to paddocks on the Fowlers Gap property, except where indicated

Site	GPS location	Trees	Branches	Domiciles (dissected)
Bald Hills 1	S 30°57'39" E 141°42'18"	27	73	721 (72)
Bald Hills 2	S 30°58'08" E 141°43'11"	6	7	28 (27)
N. Holding	S 31°04'50" E 141°41'43"	1	2	18 (12)
Sandstone	S 30°59'13" E 141°40'57"	8	9	61 (34)
N. Mandelman	S 31°00'09" E 141°54'13"	1	1	10 (0)
Connors	S 31°04'09" E 141°42'40"	13	32	194 (41)
Lake View*	S 31°56'13" E 141°30'08"	2	6	35 (35)

*Site located approximately 3 km southwest of Broken Hill, NSW.

defined distinct 'branches' as being separated by at least 1 m of contiguous bare bark without phyllodes, a substantial walking distance for ~2-mm thrips, as *D. aneurae* rarely flies (J. D. J. Gilbert, pers. observ.).

We compared the angular distribution of inhabited branches with that of all branches using a chi-squared test on the frequency of branches in each 20° interval. We looked for a directional trend in the frequency of domiciles on each branch using a generalized linear model (GLM) with 'domiciles per branch' as a response, 'sin(bearing)' and 'cos(bearing)' as predictors, and a quasi-Poisson distribution to correct for overdispersion.

To assess reproductive synchrony, we analysed the distributions of immature stages among branches, trees and sites. To count immature stages, domiciles were dissected under a binocular microscope using fine forceps by peeling away part of the domicile wall so as not to disturb live inhabitants; any interactions (e.g. mating, aggression, domicile repair, etc.) among live thrips inside the domicile were observed for a few minutes. Adult thrips, eggs and offspring of each stage (instar I, instar II, propupa, pupa I, pupa II, alate) were counted. Foundresses had lost their wings, and so were distinguishable from adult offspring (Morris *et al.*, 2002), although a few offspring may have become dealate inside the domicile (Bono & Crespi, 2006, 2008). To determine whether the distributions of immature stages within domiciles were similar among sites, trees or branches, we used a multivariate Adonis test in the vegan package in R (Oksanen *et al.*, 2008), based on pairwise Bray–Curtis distances as a measure of dissimilarity. We fitted

'site', 'tree' and 'branch' as predictors, with 'date dissected' as a covariate.

Protandry, or the development of males before females, was assessed by regressing the proportion of mature male offspring against the proportion of the brood that was mature. We used a GLM with binomial error structure, arcsin-square root transforming the predictor variable. Under protandry, we would expect this relationship to be negative.

We counted the phyllodes used in each domicile, measured their dimensions and compared these dimensions with published descriptions of *A. aneurae* varieties. We tested for an advantage to the utilization of terminal phyllodes by comparing the productivity in domiciles with and without terminal buds, using nonparametric Wilcoxon tests. Productivity was defined as the number of offspring present plus the number of unhatched eggs. Hatched eggs could not be counted because they had usually been aggregated into middens (concentrated areas of waste material; see Results). We excluded domiciles with only adult offspring because some may have dispersed, and domiciles with only eggs, because some eggs may not yet have been laid.

MATING

All adult thrips extracted from domiciles were kept in the laboratory in cages consisting of a 40-mm Petri dish with its base inverted and pressed into the lid, creating a thin disc-shaped space inside, approximately the size of a large domicile (Fig. 1). Parafilm® was sandwiched between the halves of the Petri dish

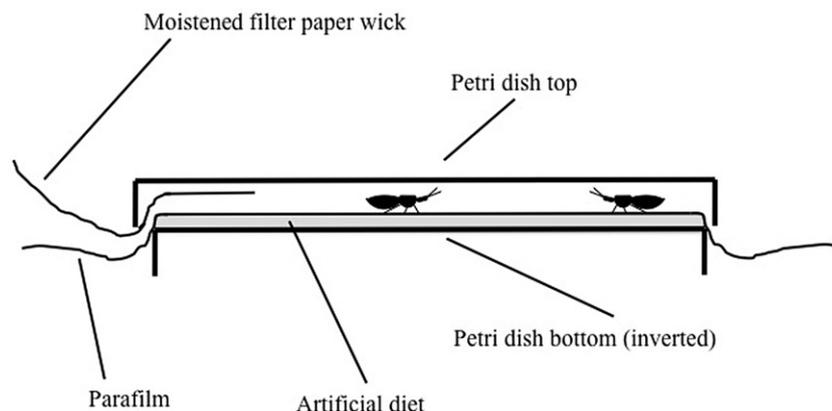


Figure 1. Apparatus used to house field-collected *Dunatothrips aneurae*.

with a droplet of liquid diet on one side and the thrips on the other. We perforated the Parafilm approx. 5 times with micropins, without which thrips failed to feed (J. D. J. Gilbert, pers. observ.), possibly through lack of volatile feeding cues. The liquid diet was adapted from that formulated by Singh (1983), and consisted of a solution of hydrolysed yeast and sucrose made up to a protein : carbohydrate ratio of 1 : 8 at 90 mg mL^{-1} , with nipagen to discourage mould. The diet was replenished every 4 days. The effects of this and other artificial diets will be dealt with in a forthcoming paper. A thin wick of filter paper was extended into the space containing the thrips and periodically moistened to keep humidity levels high, a prerequisite for survival in this species (J. D. J. Gilbert, pers. observ.) and in other thrips (Kakei & Tsuchida, 2000).

Mating trials were conducted using adult offspring extracted from field-collected domiciles maintained in Petri dishes as described above ($N = 15$). Although within-domicile matings were uncontrolled, we were confident that these individuals had not mated outside their natal domicile and had not attempted to build domiciles, because these latter activities usually happen after dispersal. In each trial, we placed a male and a female together in a cage and recorded any courtship, mating and associated behaviours that occurred. If they had not mated after 2 h, we terminated the trial. If the individuals mated, they often mated repeatedly, and so we observed them until 2 h had elapsed without a further mating. After these trials, mated individuals were used in dispersal and domicile initiation trials. We also recorded matings by naturally dispersing males (see below).

DISPERSAL AND DOMICILE FORMATION

Dispersal and domicile formation were investigated in two ways: (1) after mating trials, releasing mated females onto branches in the field and laboratory

under various treatments; and (2) watching individuals disperse naturally from mature domiciles maintained in the laboratory.

First, following mating in the laboratory, we conducted observations of mated alate females under various treatments (see Table 2 for sample sizes). In treatment A, we released females singly onto branches in the field. We used two *A. aneurae* trees at the N. Mandelman and Bald Hills sites for this purpose, and each trial employed an adjacent branch to one on which domiciles had been found and collected previously to ensure (as far as possible) a suitable location with nesting sites still available. We released females directly onto terminal phyllodes using a hair probe, subsequently checking them every 10 min. Although marking individuals was impractical, we were able to follow the movements of each for several hours, after which we returned daily to the release branches until the individuals had disappeared or domiciles had been initiated. In treatment B, females were released into clip cages placed over terminal phyllodes in the field under the same conditions as in treatment A, and were observed daily until domicile formation or death. In treatment C, females were released onto terminal cuttings taken from branches on which domiciles had been collected, or neighbouring branches. Cuttings were maintained in the laboratory inside inverted 10-mL pipette tips with their points immersed in a 5 mL L^{-1} broth of liquid NPK fertilizer (Aquasol®) in 10-mL vials, held in place by a rubber cap. The reservoir was changed every 4 days. Treatment D was identical to treatment C, except that the terminal phyllodes had been experimentally bunched together loosely using wire ties to minimize the cost of phyllode manipulation for the thrips. Treatment E was identical to treatment C, except that larger cuttings were used that contained a choice of 5–10 terminal buds. Treatment F used larger cuttings as in treatment E, but with terminal phyllodes experimentally bunched as in treatment D.

Table 2. Summary of treatments used to investigate conditions necessary for domicile initiation in *Dunatothrips aneurae*

	Brief description (see text for explanation)	<i>N</i>	Made domicile	Laid eggs
A	Mated alate females extracted from mature domiciles, unconfined, released onto terminal phyllodes in the field	12	0	0
B	Mated alate females extracted from mature domiciles, released onto terminal phyllodes in the field, but confined inside a clip cage	7	0	0
C	Mated alate females extracted from mature domiciles, released onto laboratory-maintained cuttings with one terminal bud	10	0	0
D	Mated alate females extracted from mature domiciles, released onto laboratory-maintained cuttings with one terminal bud, with phyllodes experimentally bunched together	6	0	0
E	Mated alate females extracted from mature domiciles, released onto larger laboratory-maintained cuttings with several (5–10) terminal buds	10	0	0
F	Mated alate females extracted from mature domiciles, released onto larger laboratory-maintained cuttings with several (5–10) terminal buds, with phyllodes experimentally bunched together	12	0	0
G	Mated pairs kept together after mating overnight before releasing onto laboratory-maintained cuttings with one terminal bud	12	10	5

In treatment G, single females were allowed to remain with males after the mating trial, and the pair was left overnight before being released together onto single-bud cuttings as in treatment C. Any domiciles initiated were observed daily, noting the extent of silk production, timing and nature of wing loss by the foundress female (as this had not been observed previously) and the timing of egg laying and hatching.

Second, we observed the behaviour of individuals dispersing in the laboratory from mature field-collected domiciles. Fifty active domiciles were collected and maintained in nutrient broth in the laboratory (as described for the cuttings above) and monitored daily. Dispersing individuals were observed in the few hours after they first emerged from a domicile and were allowed to roam over an *A. aneura* branch (with 5–10 terminal buds) placed in contact with the domicile. After emergence, we recorded the time they spent outside and inside the natal domicile, and whether they attempted to fly. Individuals were judged to have fully dispersed if they re-encountered their natal domicile (i.e. walked over the silk) without re-entering it, if they attempted to fly at any point or after 12 h outside the domicile.

To investigate male behaviour after dispersal, dispersed males ($N = 9$) were then allowed to roam onto another *A. aneura* cutting in the laboratory, placed against that on which they currently were wandering. This new cutting contained two domiciles at different stages of development: (1) a newly initiated domicile containing one or more females that had not yet laid eggs; and (2) an active domicile containing female(s) and developing offspring. Case (1) represents a better

mating opportunity than case (2) for a prospecting male, because females only produce one generation of offspring (Bono & Crespi, 2008). We recorded whether each domicile was entered, the latency to do so and any mating observed between the focal male and resident female(s). Finally, we tested whether males were attracted to the immature domicile itself. We introduced dispersing males to *A. aneura* branches containing immature ($N = 7$) and mature ($N = 5$) domiciles from which females had been removed, and recorded the latency to enter and exit the empty domicile.

DEFENCE

To assess defensive or aggressive reactions, we initially prodded individuals gently during dissection of the domicile using a probe made from a stout, flexible hair (obtained from a shaving brush), and recorded any defensive behaviours observed. To assess the reactions of the residents to conspecifics, we introduced lone foreign males and females into domiciles through small artificial holes made in the outside walls of mature domiciles ($N = 15$ domiciles). Holes are occasionally found in field domiciles but, in the laboratory, are quickly repaired by residents (J. D. J. Gilbert, pers. observ.). Again, we recorded any defensive behaviour by residents and the behaviour of the intruder over the ensuing few hours.

All statistical analyses were carried out in R 2.13.0 (R Development Core Team, 2011); mixed models were fitted using the lme4 package (Bates, Maechler & Bolker, 2011).

RESULTS

DISTRIBUTION OF DOMICILES IN SPACE AND TIME

A total of 1077 *D. anurae* domiciles was counted on 58 trees at eight sites, 221 of which were dissected for laboratory analyses (Table 1). Domiciles were distributed patchily; our searches typically covered 100–300 uninhabited trees between patches. Inhabited patches usually consisted of one to five neighbouring trees. A degree of host preference or host fidelity over multiple generations could be inferred from the fact that at least four of the trees originally surveyed in 2005 by Bono & Crespi (2008) (located using GPS coordinates) still contained active domiciles in 2011/2012.

Within inhabited trees, exhaustive searches showed that domiciles were also distributed patchily, typically clustering on one or a few branches per tree. On eight trees for which total branch architecture was recorded, a total of 61 of 161 branches contained domiciles. Inhabited branches in the whole sample followed the same angular distribution as the total branches of trees in which the entire architecture was recorded (grouping branch orientations into 20° bins, χ^2 test, $\chi^2 = 67$, d.f. = 64, $P = 0.37$), with most branches, and most inhabited branches, occurring around 90°, or east facing (Fig. 2). Inhabited branches contained a median of three domiciles \pm standard

deviation of 17.4 (range, 1–122). Approximately east-facing branches contained slightly more domiciles (Fig. 2; GLM with ‘sin(bearing)’ and ‘cos(bearing)’ as predictors, quasi-Poisson distribution: $\chi^2 = 41.6$, d.f. = 1, $P < 0.01$; Fig. 2).

The distributions of immature stages in domiciles differed among sites (including only branches with three or more domiciles; Adonis test, $F_{4,84} = 2.07$, $P = 0.001$) and among trees ($F_{9,84} = 1.46$, $P < 0.05$), but not among branches ($F_{4,84} = 0.89$, $P = 0.65$), even though, in a separate model, ‘branch’ on its own explained significant variation (Adonis test, $F_{16,84} = 1.68$, $P < 0.005$). The covariate, date dissected, was also significant in the full model ($F_{1,84} = 4.23$, $P = 0.001$). Thus, although domiciles within a branch were not distinct in the distribution of immature stages, trees were distinct from each other, as were sites, suggesting an intermediate degree of reproductive synchrony.

We found no evidence for protandry in field broods; the proportion of male alates actually increased slightly with the proportion of the brood that had matured, indicating mild protogyny (GLM, binomial errors, $\chi^2 = 6.51$, d.f. = 1, $P = 0.01$).

Founding males were rarely found in the domicile: in mature field domiciles without alate offspring (assessed by the absence of alate females; $N = 105$ excluding one domicile with 34 male offspring), alate

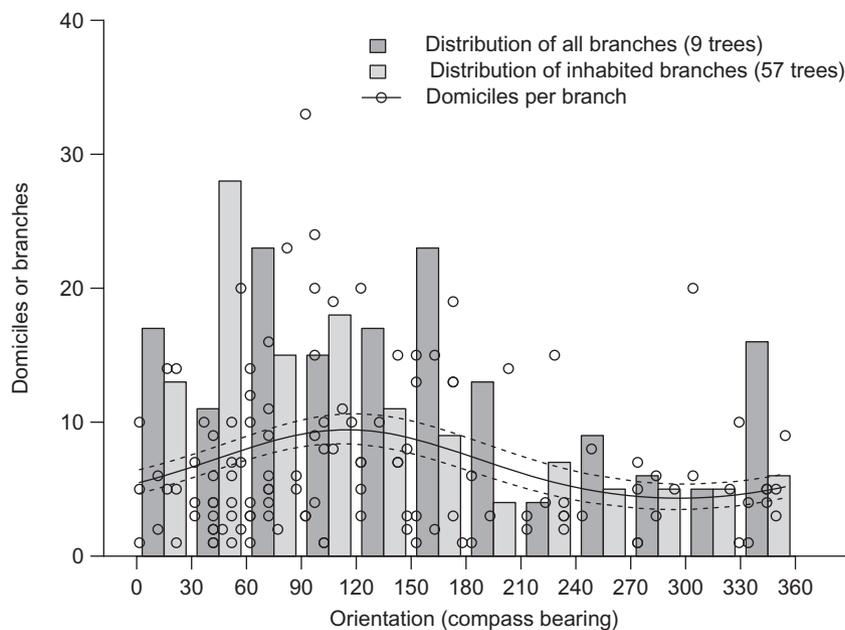


Figure 2. Number of *Dunatothrips anurae* domiciles on a branch with respect to orientation (compass bearing) on the tree. A degree of scatter has been applied to separate overlapping points. Line shows best-fit regression line (backtransformed from generalized linear model); broken lines show confidence intervals. Distribution of inhabited branches is given for comparison, together with the distribution of all branches on trees for which the entire branch architecture was recorded.

males were rarely seen ($N = 20$ males from 12 domiciles), occurred singly in only eight domiciles, and a dead, single male occurred in only one domicile. Dealate males were found in only two of 221 domiciles.

Dunatothrips aneurae has been reported to prefer thin-phyllode varieties of *A. aneura* (Crespi *et al.*, 2004) and, within these, terminal phyllodes (Morris *et al.*, 2002). *Acacia aneura* is a large species complex whose phyllodes are variable in size and shape, described as ranging in length from 25 to 125 mm and in width from 0.8 to 9 mm (World Wide Wattle Dataset, <http://www.worldwidewattle.com>). Phyllodes utilized in *D. aneurae* domiciles were not exceptional in length (phyllode length used in domiciles, 0.9–101.9 mm; mean, 41.6 mm), but were unquestionably among the narrowest exemplars of the species (phyllode width used in domiciles, 0.55–2.6 mm; mean, 1.68 mm). Varieties of *A. aneura* with cylindrical or straight terete phyllodes (e.g. *A. aneura* var. *aneura* and *A. aneura* var. *microcarpa*; Miller, Andrew & Maslin, 2002) were more likely to harbour *Dunatothrips* domiciles than those with flat or broad phyllodes (J. D. J. Gilbert, pers. observ.). The majority of assessed domiciles (76.5%, $N = 153$) encompassed an apical bud, suggesting a preference for terminal phyllodes. However, this did not appear to translate into greater reproductive success for domiciles containing apical buds (Wilcoxon test: productivity per domicile: $W = 1604$, $N = 139$, $P = 0.30$; productivity per foundress: $W = 1552$, $N = 130$, $P = 0.76$).

MIDDENS

Most mature domiciles contained a midden (Fig. 3), i.e. an area of the domicile in which eggshells, exuviae, detached wings and dead bodies of nestmates were concentrated. In rare cases, there were up



Figure 3. Midden of *Dunatothrips aneurae* domicile.

to three middens. Domiciles containing a midden had more foundresses than those without (median with midden, 2.0; range, 1–11; median without midden, 1.5; range, 1–5; Wilcoxon test, $W = 1184$, $N = 84$, $P < 0.001$). Several observations suggested that waste material had probably not accumulated passively as a result of gravity: domiciles were typically horizontally oriented, middens were usually highly compacted and covered in silk of a similar appearance to the domicile wall, and, outside of middens, eggs were generally stuck to the wall, preventing them from falling. Probably, the waste items had been actively carried there by domicile members, although this behaviour was not observed.

MATING

In total, mating was observed from start to finish 38 times. In mating trials in a Petri dish, the male sometimes antennated the female's body, then turned through 180° and began antennating the female's genital region whilst rubbing the end of his abdomen on her antennae. This lasted a median of 12 s (range, 10–20 s; $N = 12$) and may be involved in courtship, or sex, species or kin recognition. Males and females are of similar length and mating required the male to twist his abdomen sideways, which was not always possible, depending on the relative sizes of male and female; one large male was seen twice to fail to mate with small females. Reproductively mature females (both mated and apparently unmated) often resisted male advances by arching and twisting their abdomen, making it impossible for the male to couple. After mating, the pair separated, but occasionally ($N = 3$ in Petri dish trials) remained in proximity, mating up to seven times, with subsequent matings typically shorter than the initial mating. There was no obvious mate-guarding behaviour of any kind. Multiple mating was observed in the Petri dish by four males (each with up to three females) and three females (each with two males) when given sequential partners, and so multiple mating is not rejected by either sex, but it is not yet known how many times females and males typically mate in nature. Neither nestmate nor non-nestmate males showed any aggression towards each other, even with females present, although frequent misguided attempts at mating with other males were observed, a phenomenon also seen in *Idolothripines* (B. Kranz, pers. comm., University of Sydney, Sydney, Australia).

Male and female offspring from the same domicile often mated with each other, both in the Petri dish and in their natal domicile. Offspring left alone to mature initiated domiciles in the laboratory four times, suggesting that inbreeding had occurred. Furthermore, we confirmed the occurrence of true

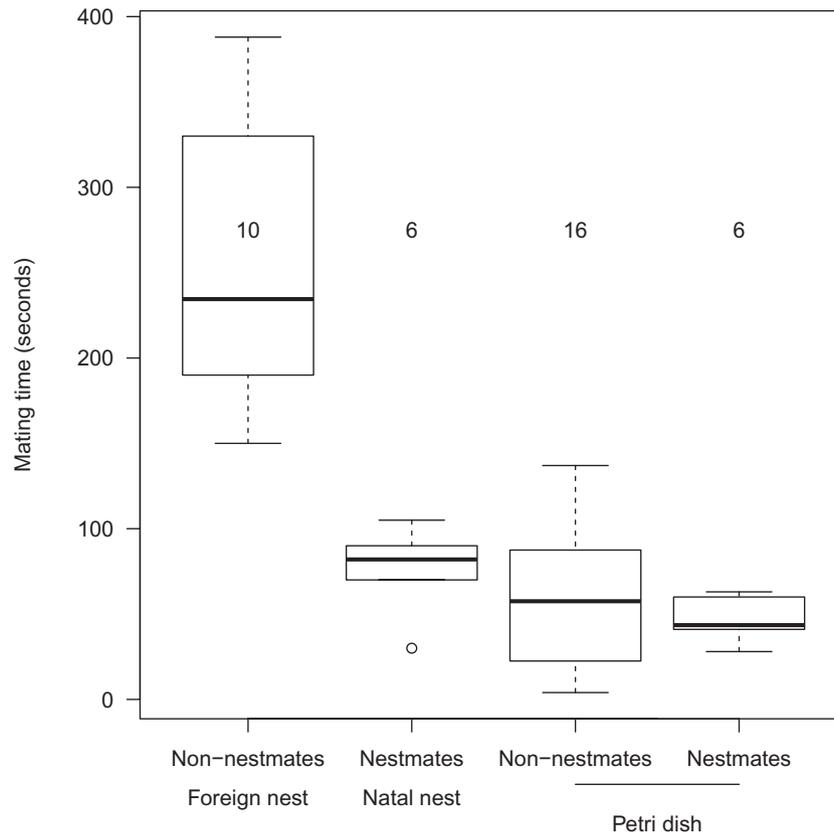


Figure 4. Mating duration [median \pm interquartile range (IQR), boxes; 90% confidence intervals (CI), whiskers] between *Dunatothrips aneurae* nestmates and non-nestmates in different environmental contexts. Sample sizes are given for each box.

inbreeding, rather than mating among different matrilineal lines in co-founded domiciles; on two occasions, males and females within single-foundress domiciles were observed repeatedly mating prior to dispersal.

The duration of mating depended on the context (i.e. 'natal domicile', 'foreign domicile' or 'Petri dish'), but not on whether the pair were actually nestmates (linear mixed-effects model with 'context' and 'whether nestmates' as predictors and 'pair ID' as a random effect; dropping 'context', likelihood ratio test, $\chi^2 = 21.20$, d.f. = 1, $P < 0.001$; dropping 'whether nestmates', $\chi^2 = 0.14$, d.f. = 1, $P = 0.74$; Fig. 4). In the Petri dish, mating lasted a median of 60 s whether or not the pair were nestmates (range, 15–137 s; $N = 22$). Inside the natal domicile, mating lasted a median of 74.5 s (range, 30–105 s; $N = 6$). Inside a foreign domicile (after males had dispersed), mating lasted a median of 234.5 s (range, 150–388 s; $N = 10$). Excluding matings in foreign domiciles, there were no differences in mating times among treatments (dropping 'context', likelihood ratio test, $\chi^2 = 1.57$, d.f. = 1, $P = 0.21$; dropping 'whether nestmates', $\chi^2 = 0.46$, d.f. = 1, $P = 0.49$).

On three occasions in foreign domiciles, females became agitated after mating repeatedly with immigrant males, and evicted them from the domicile using head butts and tail flicks.

DISPERSAL AND DOMICILE FORMATION

Alate female *D. aneurae* were never, and to our knowledge have never been, observed flying. Rather, mated alate females released singly onto host plants (whether in the field, $N = 12$, or in the laboratory, $N = 38$) wandered up and down along the length of each phyllode on a branch several times, turning and retracing their steps after encountering woody tissue for more than approximately 10 cm. In the laboratory, wandering continued for up to 3 days, but these females were never seen to initiate a domicile alone. In the field, females released unconfined ($N = 12$, treatment A in Table 2) were rarely resighted (only three were seen the next day; none remained after 48 h). Alate males were also almost never observed to fly; of the 90 domiciles dissected in the laboratory that contained alate offspring, males from only one

domicile flew, directly after mating in the laboratory. It is not known what triggered this behaviour, but it demonstrates that flight is possible by at least some alate male *Dunatothrips*, and its importance in dispersal remains to be tested.

In the domiciles from which permanent dispersal was observed directly ($N = 5$), male ($N = 10$) and female ($N = 8$) nestmates dispersed on the same day; males often exited and re-entered several times before permanently dispersing, whereas females tended to disperse once, permanently, although a few short forays were observed. Typically, males made several short forays around their natal domiciles, returning each time ($N = 10$ males, four of which re-entered the domicile; range, 1–7 forays; duration, 3–283 min).

Of nine permanently dispersed males given a choice, all chose to enter the immature domicile over the mature domicile ($\chi^2 = 9.0$, d.f. = 1, $P < 0.01$). The silk of mature domiciles was antennated, but males did not attempt to enter. On encountering an immature domicile, males immediately sought to squeeze through a gap in the silk. Based on this finding, we hypothesized that males were attracted to the newly formed domicile itself, rather than the reproductive phase of the females inside, and so we introduced another set of dispersed males to immature and mature domiciles whose females had been removed. All seven males entered and remained inside the empty immature domiciles (duration, 20 min to 23 h), whereas only one of five males entered the empty mature domicile (duration, 25 min) (Fisher's exact test, $P = 0.01$).

Alate female *D. aneurae* were only seen permanently dispersing from two domiciles; unlike males, they rarely returned to the natal domicile. From these two domiciles, several females, both alate and dealate, emerged within a few hours of each other (domicile 1: $N = 3$ alates, $N = 2$ dealates; domicile 2: $N = 2$ alates). Whether the dealate individuals were foundresses or dealate offspring is unknown. The females did not obviously interact and there was no indication that females followed trails left by earlier dispersed females, as in co-founding spider mites (Yano, 2008). The idea that dispersing female nestmates may coordinate prospecting behaviour, a possible precursor to domicile co-founding (Bono & Crespi, 2008), requires further confirmation. Three further females made brief forays outside other domiciles (range, 5–59 min). Dealate females were also seen making forays outside active domiciles that were not yet at the dispersal stage ($N = 12$ females; range, 5–61 min).

Females never built domiciles if they were kept alone without mating. Furthermore, laboratory trials suggested that, even once mated, a female still

required the presence of a male beyond the initial mating in order to initiate a domicile. Once-mated females would not nest if they were subsequently isolated, but readily nested when paired with a male overnight before both were released together onto cuttings in the laboratory. Of the treatments we applied, only continuous male presence after the first mating resulted predictably in successful domiciles (Table 2).

Domiciles were created by stretching a sticky silk-like secretion from the end of the terminal abdominal segment between adjacent phyllodes. Males were never seen helping in this process, whether constructing domiciles in the laboratory ($N = 9$) or in numerous laboratory observations of domicile repair (J. D. J. Gilbert, pers. observ.). We estimated silk density each day, as an approximate proportion of the density observed in completed field domiciles (opaque with few visible gaps; cf. Crespi *et al.*, 2004: 8, and see wall silk in Fig. 3), in increments of 0.1. Not all laboratory domiciles reached complete silk density. In the nine domiciles observed, silk was laid down at varying rates; domicile construction was terminated at varying times (5–12 days after initiation; median, 12 days) and varying silk densities (0.1–1; median, 0.75). Egg laying was seen in seven domiciles and began 15–26 days (median, 20 days) after domicile initiation (3–21 days after domicile completion; median, 8 days). In domiciles in which more than one batch of eggs was laid, the median interval between egg batches was 4 days (range, 3–5 days). Eggs were laid at a median rate of one per day (range, 0.5–2) and females laid a median of two eggs (range, 1–7). In the laboratory, most domiciles were abandoned after egg laying, probably because of mould, and only four eggs hatched; for these, the hatching time was 15–16 days (median, 16 days). These data should therefore be treated with caution until the species can be reared successfully in the laboratory.

DEALATION IN FOUNDRESS FEMALES

No apterous or brachypterous offspring were ever observed from field broods. However, alate females, but not males, invariably lost their wings during domicile formation in the laboratory. This occurred by a process of autogenous abscission: the wing hinged along a sharply defined line of weakness (Fig. 5). Thus weakened, the wing appeared to require mechanical force to pull it off. This was effected by rubbing the wings against the silk, which was observed twice; detached wings were usually found stuck to the domicile wall when not incorporated into the midden. In one case, the wings remained attached for 8 days.

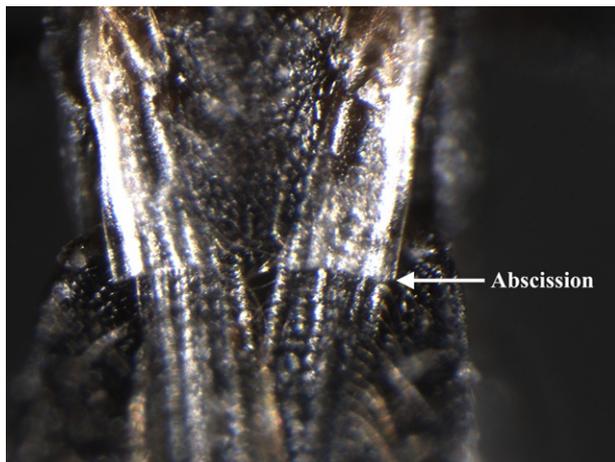


Figure 5. Detail of wings of foundress *Dunatothrips aneurae* in the process of becoming dealate within the domicile.

DEFENCE AND REACTION TO CONSPECIFICS

During domicile dissection, we observed no obvious defensive behaviour from inhabitant thrips. Individuals of all stages required considerable tactile stimulus before reacting, and the reaction consisted only of evasive walking. The foreleg tubercle (spur), which occurs on both sexes, and has been suggested to be defensive weaponry (Morris *et al.*, 2002), was never used.

Foreign individuals introduced to mature domiciles uniformly entered through the artificial hole made in the silk wall. Adult thrips inside the domicile moved agitatedly whilst the foreigner attempted to enter, but, once the conspecific had entered, there was no aggression and the intruder was not molested, whether male or female. Although it was impractical to mark the conspecific, we followed foreign conspecifics for up to 3 h after entry, and none was attacked in that time or left the domicile.

DISCUSSION

DISTRIBUTION OF DOMICILES IN SPACE AND TIME

The patchy distribution of *D. aneurae* suggests limited dispersal, supported by preliminary genetic data (Bono & Crespi, 2008) and by reproductive synchronization of domiciles within trees and sites. Such population viscosity could bring about the conditions for the evolution of co-founding behaviour via kin selection (although see section on Defence), depending on how frequently long-distance dispersal occurs (Wilson, Pollock & Dugatkin, 1992). The most likely candidate dispersal mechanism is by wind, as part of the 'aerial plankton' (Lewis, 1973; Taylor, 1974). Aggregation on east-facing branches may be because

of the prevailing winds or may occur actively, for example, to maximize sun exposure or exploit orientation-specific branch chemistry. How new patches are colonized and how trees or varieties are chosen remain unknown. *Dunatothrips aneurae* may fail to establish domiciles on broad-phyllode varieties for mechanical reasons: phyllodes of these varieties tend to be relatively stiff and widely spaced. *Dunatothrips aneurae* may be especially productive on some host genotypes, or some varieties may be resistant to attack by *D. aneurae*. Within a tree, domiciles containing terminal buds were not especially productive, and so this preference may not be nutritional as suggested by Morris *et al.* (2002), although offspring quality (e.g. egg size) was not assessed; alternatively, terminal phyllodes may be more closely bunched, requiring less silk to form a domicile.

The absence of founding males in field domiciles supports previous observations (Morris *et al.*, 2002; Bono & Crespi, 2006, 2008) and suggests that founding males either leave to seek mates elsewhere, or are evicted; three anecdotal observations in the laboratory supported the latter hypothesis. It should be noted that the feeding mechanism of thrips (a piercing stylet) means that, if males were eaten by females after mating, male remains would be evident within the domicile, or, when dissected, in the midden, but this was rarely seen.

MIDDENS

Among eusocial insects, waste localization is common (Weiss, 2006; Jackson & Hart, 2009); in non-eusocial insects, it is often observed in insects feeding in enclosed shelters, especially those in family groups (reviewed by Weiss, 2006). In a sealed environment, such as a *Dunatothrips* domicile, middens may play a hygienic role by sequestering decaying material, similar to burial behaviour in claustral termites (Chouvenc *et al.*, 2012), reducing pathogenic infection and reducing potential olfactory cues to predators, or may free up space (see Weiss, 2003). If so, middens might be expected to be a source of microbial or fungal pathogens. In a study of defences against pathogens, Turnbull *et al.* (2010) found no cuticular antimicrobial activity in *D. vestitor*, a domicile builder with similar ecology to *D. aneurae* (Crespi *et al.*, 2004). This generates an interesting hypothesis: *Dunatothrips* silk, particularly that covering middens, may have antimicrobial properties. Antimicrobial properties of silk have been shown in some taxa (Korayem *et al.*, 2007; Li *et al.*, 2007), but not in others (Fountain & Hughes, 2011).

MATING

Both inbreeding (sibmating) and outbreeding (in foreign domiciles) were observed, before and after

dispersal, respectively, and their relative importance remains to be clarified. Preliminary genetics suggests that inbreeding is not a strong evolutionary force in this species (Bono & Crespi, 2008). Lack of protandry in *D. aneurae* may also indicate that sibmating is relatively unimportant, and is consistent with males exploiting outside mating opportunities after sibmating (see section on 'Dispersal'). However, population viscosity would increase relatedness among and within domiciles, reducing the relative disadvantage of inbreeding, at least locally; moreover, local mate competition caused by inbreeding (Hamilton, 1967) is also consistent with the female-biased sex ratio observed by Bono & Crespi (2008). The origin of sociality in *Kladothrips* has been associated with inbreeding (Chapman *et al.*, 2000). It would be interesting to investigate factors affecting inbreeding in *D. aneurae*, including cues of outside options, such as domicile density (Kokko & Ots, 2006), whether females preferentially choose males from unrelated matrilineal lines when breeding within the natal domicile and whether co-founding females compete over immigrant males.

Mating in foreign domiciles took longer than in natal domiciles, which may reflect reduced investment in inbreeding; male spiders sometimes mate for longer when outbreeding than inbreeding (Welke & Schneider, 2010). In the Petri dish, mating was no different between nestmates and between non-nestmates, and so males may use a cue from the domicile itself to assess relatedness to females. Silk may carry information about genetic relatedness (Clotuche *et al.*, 2012), or, perhaps more likely, volatile pheromones may indicate domicile age (Yano, 2008). Alternatively, dispersal itself may trigger increased investment in mating. Conceivably, females may be in control of mating duration, allowing immigrant males to mate for longer than brothers. However, usually only the longest matings were associated with female resistance, which was generally rare.

That some females mated multiply is especially interesting, because recent studies have suggested that strict genetic monogamy is an important prerequisite for the origin of sociality (Boomsma, 2007, 2009), with support from many taxa (Cornwallis *et al.*, 2010; Lukas & Clutton-Brock, 2012), including the haplodiploid Hymenoptera (Hughes *et al.*, 2008). If one's mother was genetically monogamous, one is equally related to siblings and offspring; raising either one carries identical genetic payoffs, and any ecological benefit of helping siblings will favour social evolution. This applies even more strongly in joint-nesting species where putatively cooperating individuals would be raising nieces, not sisters. Thus, the coexistence in *D. aneurae* of co-founding behaviour with potential female promiscuity, which dilutes

within-nest relatedness, warrants further study. Females may mate multiply to gain direct benefits (e.g. Wagner *et al.*, 2001) or, alternatively, for indirect benefits, by reducing variance in fitness via bet-hedging (Fox & Rauter, 2003), or by increasing the chance of finding a male who is of good quality (Fedorka & Mousseau, 2002), compatible (Zeh & Zeh, 1996, 1997), or good at sperm competition (Keller & Reeve, 1995).

DISPERSAL AND DOMICILE CONSTRUCTION

A female *D. aneurae* required male presence for extended periods beyond the first mating in order to initiate a domicile – whether for physical assistance, repeated mating (Fox, 1993), nutritional gifts (Wagner *et al.*, 2001) or something else requires further research. Given their small number of eggs, it is highly unlikely that *D. aneurae* are sperm limited. As thrips are haplodiploid, however, we may expect that virgin females, or even those whose sperm stores were exhausted, could still lay male eggs – as do worker Hymenopterans routinely. In *Kladothrips* spp., all-male broods range from rare (Kranz *et al.*, 1999, 2001) to nearly 25% in a species with a split (i.e. bimodal) sex ratio (Kranz *et al.*, 2000). However, of 221 *D. aneurae* domiciles dissected, only one single brood contained all males – a large brood of 34 males. Bono & Crespi (2008) found no all-male broods in their sample of over 500 domiciles.

Two observations suggest that silk production may be costly for females. First, variation in the rate of silk deposition in the laboratory suggests that domicile building may represent a substantial expenditure. Second, silk and egg production were well separated in time, suggesting that these tasks cannot be performed together and may trade off against each other. Multiple females may construct domiciles more rapidly than single females, which requires confirmation.

Finally, the fact that dispersing males appeared to be searching for already initiated domiciles suggests that these are not the males that are required at the moment of domicile initiation by females – potentially, females may co-disperse with brothers, although this was not observed.

DEALATION IN FOUNDRESS FEMALES

Lack of aptery or brachyptery rules out wing diphenism or dimorphism. Wing loss did not arise from mutilation by nestmates, as in some termites (Zimmerman, 1983; Myles, 1986; Roisin, 1994). The sharpness of the wing abscission line suggests that it may begin with cell autolysis. Dealation occurs in at least eight orders of insects (Myles, 1988). In *D. aneu-*

rae, detached wings are not eaten or otherwise utilized, but dealation may allow muscle histolysis and digestion, as in crickets (Tanaka, 1994) and ants (Davis, Jones & Farmer, 1989), and may also trigger oviposition (Jemielity, Gräff & Keller, 2006).

DEFENCE AND REACTION TO CONSPECIFICS

Dunatothrips aneurae did not react defensively to either conspecifics (this study) or inquilines (Gilbert, Mound & Simpson, 2012). Evolutionary suppression of aggression towards conspecifics is thought to facilitate pleometrosis in some systems (Miller & Crespi, 2003), and will be an interesting focus for future study. *Kladothrips*, which are monogynous, show lethal aggression towards same-sex conspecifics (Crespi, 1992). Other populations of *D. aneurae* are affected by kleptoparasitic *Xaniothrips mulga* (Bono & Crespi, 2006; Bono, 2007), however, and so we cannot be certain that defence is entirely absent.

Being haplodiploid, female thrips are related to full sisters by 0.75, and thus might be expected to have strong, not weak, mechanisms of kin preference (although female promiscuity weakens this expectation). In general, social groups that have evolved primarily via kin selection tend to display high degrees of interfamilial aggression, and its absence is seen as a paradox, e.g. in unicolonial ants (Queller & Strassmann, 1998). In contrast, species that nest gregariously or communally (e.g. tent caterpillars, Costa & Ross, 2003; aphids, Akimoto, 1981; Miller, 1998a, 2004) commonly show a lack of aggression among genetically distinct lineages, presumably favoured by direct fitness advantages of group size (Costa & Ross, 2003) or genetic diversity (Liersch & Schmid-Hempel, 1998; Page & Erber, 2002). Thus, future work should aim to clarify the roles of indirect (kin-selected) versus direct benefits of co-founding in *D. aneurae*, as well as intraspecific aggression in less cooperative *Dunatothrips* species (e.g. *D. gloius* never cooperates; Bono, 2007).

CONCLUSION

Taken together, these data are largely consistent with the scenario deduced by previous authors: *D. aneurae* has limited dispersal, resulting in locally dense, reproductively synchronized patches of domiciles, although longer distance dispersal must occur at least occasionally – probably via the aerial plankton. Evidence is mixed over whether limitation of breeding sites favours cooperative behaviour in insects (see Brockmann, 1997) – this may be the case for *D. aneurae* (J. D. J. Gilbert & S. J. Simpson, unpubl. data), but does not fully explain the extent of co-founding (Bono & Crespi, 2006). Our observations suggest several

other factors at play that may also hold key insights into the evolution of pleometrosis, and which should now be a focus for future study. First, suppression of aggression is a factor implicated in the evolution of co-founding (see above). Second, an absolute female requirement for male presence at the moment of nest building would link nesting opportunities to the sex ratio, which is female biased (Bono & Crespi, 2008). Breeding opportunities may thus be a scarce resource for females and they may consequently be forced to cooperate. Alternatively, cooperating with other females may circumvent the requirement for a male, if this requirement stems from nutritional or energetic costs of nest building. Third, although reproductive skew is unknown in *D. aneurae*, two observations suggest potential division of labour within a domicile – middens suggest that domiciles require active maintenance, and the potential trade-off between silk and egg production (e.g. Craig *et al.*, 1999) suggests that these two tasks may be inequitably distributed. In the apparent absence of defensive behaviour (otherwise a classic role for subordinates, including thrips; Crespi, 1992), division of labour with respect to domicile construction and maintenance should be a focus for future work in *D. aneurae*.

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