Sex-specific dispersal behaviour of crawlers in the mealybug
Planococcus citri

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ABSTRACT

Sex-specific dispersal can have important evolutionary and ecological implications, influencing local population structure and sex ratio, as well as the speed at which new habitats can be colonized. In scale insects, first-instar nymphs (crawlers) are assumed to be the main dispersal stage. Although all scale insects are extremely sexually dimorphic, in most species the sexes are indistinguishable as crawlers. Here we consider the mealybug Planococcus citri (Risso), and dispersal by crawlers to or from resource patches. The aim of this study was to test if: (1) crawler dispersal behaviour differs between the sexes and how this is affected by local conditions (population density and sex ratio); and (2) there is a difference between the sexes in crawler dispersal success to a new host plant. Using two experiments, which differed in how resources were spread between dispersal sources and sinks, we show that male and female nymphs do not differ in their dispersal behaviour or in their dispersal success when dispersal is via crawler locomotion. These laboratory experiments are an important starting point for understanding the evolution of dispersal behaviour of P. citri in the field, suggesting that more attention might need to be paid to different methods of dispersal as well as crawler locomotion.

KEYWORDS: Mealybug, sex-specific dispersal, crawler behaviour, sex ratio.

Introduction

Patterns of dispersal are important factors shaping both population structure and the opportunity for and speed of colonisation of new habitats (Bullock et al. 2002). There has been substantial work on the dispersal behaviour of scale insects. Many studies have identified that the juvenile stages are the main agents of dispersal (Beardsley and Gonzalez 1975, Greathead 1990, Greathead 1997, Gullan and Kosztarab 1997). First-instar nymphs (crawlers) have been found to possess numerous characteristics that have been considered adaptations for dispersal behaviour, including long legs and antennae (relative to later instars: Beardsley and Gonzalez 1975, Gullan and Kosztarab 1997). Additionally, dispersal strategies may be more passive, and crawlers of several species have been found to exhibit behaviours that increase the chances of wind dispersal and to use wind dispersal to migrate for several
kilometres (Washburn and Washburn 1984). Crawlers from several species have also been found to be able to survive without food for extended periods, which should again enhance their dispersal success (Gullan and Kosztarab 1997).

Several studies have attempted to estimate crawler dispersal rate and distance under both laboratory and field conditions, as well as to establish the factors influencing crawler dispersal (reviewed by Greathead 1990, Greathead 1997). However, experimental tests are rare (but see Washburn and Frankie 1981, Washburn and Washburn 1984). As such, at least one aspect of the crawler dispersal behaviour has yet to be seriously addressed: the potential difference in dispersal behaviour between the sexes during the crawler stage. If there is such a difference, this would be important as it will alter the predicted effects of dispersal rates on colonization of new habitats and on the population structure. For example, sex-biased dispersal might change the local operational sex ratio, with the source population being biased towards the least migratory sex, while newly established populations will be biased towards the migratory sex (Leturque and Rousset 2003). However sex-biased dispersal can also affect sex ratio in a different way. Since related crawlers might compete locally for resources where they hatch, if one sex has a lower dispersal rate than the other, then this sex is expected to experience higher levels of kin competition (Hamilton 1967). This in turn can lead to biased primary sex ratios as ovipositing females are expected to bias the sex ratio of their offspring they produce in order to minimize competition among their offspring (Hamilton 1967, Clark 1978, West 2009). Indeed, primary sex ratio adjustment to local density has been observed in the mealybug Planococcus citri (Varndell and Godfray 1996, Ross et al. 2010). Additionally, recent theoretical work has shown that sex ratio and sex-biased dispersal can co-evolve, and that biased sex ratios can select for sex-biased dispersal and vice versa (Leturque and Rousset 2003, Leturque and Rousset 2004).

But why would there be differences between dispersal behaviour of male and female crawlers? Male and female scale insects differ in several important ways. Firstly, males stop feeding after the second instar. Because of this they need fewer resources, and need them for a smaller proportion of their life. Therefore they are potentially less sensitive to both resource competition and food shortage compared to females and might be less inclined to risk dispersion under those circumstances. Secondly, adult males are winged while adult females are almost completely sedentary in most species, and males also have a very sensitive pheromone receptor system and are able to find females at large distances (Branco et al. 2006). This suggests that young males do not need to disperse to find mates. Based on these two factors, we predict that female crawlers are more likely to disperse than male crawlers. Although there has yet to be an experimental study on sex-specific dispersal in scale insects, there are suggestive observations of a few species in which there is a morphological difference between the sexes at the early juvenile stage (Brown and Bennett 1957, Greathead 1990, Gullan and Kosztarab 1997). However, all these species are exceptional in that their crawlers are sexually dimorphic. The dimorphism has allowed easy observation of behavioral differences between the sexes, but this might not be representative for other scale insects, as the dimorphism might have evolved as a result of the differential behaviour specific to these species. It is therefore important to test for sex-specific crawler dispersal in species that do not show sexual dimorphism at the crawler stage (as is the case in the large majority of species).

Usually crawlers are expected to settle close to where they were born (Beardsley and Gonzalez 1975, Nestel et al. 1995, Gullan
and Kosztarab 1997), possibly because dispersal is risky. However, presumably local conditions will influence dispersal behaviour under some circumstances (Hamilton and May 1977, Comins et al. 1980). There are two situations when dispersal might be selected for: (1) high local density, as this might result in high levels of competition (both between kin and between unrelated individuals); (2) local exhaustion of resources (e.g. due to death of the host plant) (Washburn and Washburn 1984). Based on the predictions described above we expect that female crawlers are under stronger selection pressure than males to disperse in order to avoid the adverse effects of crowding and lack of resources.

In this paper we present the results of two experiments that test for sex-specific differences in dispersal rate, addressing both crowding and resource availability and the associated effects on mortality. The first experiment tested the effect of crawler density and sex ratio on the dispersal probability of both sexes in a fully factorial design with two density treatments and two sex ratio treatments (male biased versus female biased). The second experiment tested for a difference in dispersal success between males and females when there was no local food source, again considering two sex ratio treatments (male biased versus female biased). We also consider the role of differential mortality, by comparing both primary sex ratio (determined at the egg stage) and secondary sex ratio (determined at the third instar).

Materials and Methods

Study system

The experiments were conducted using the citrus mealybug Planococcus citri (Risso) (Hemiptera: Coccoidea: Pseudococcidae). This cosmopolitan species can parasitize a wide variety of host species and is easily cultured in the laboratory P. citri has strictly sexual reproduction and an unusual genetic system in which the chromosomes inherited from the father are deactivated (condensed) during early development in males and not passed on in the germ line (Schraed 1921, Schraed 1922, Brown and Nelson-Rees 1961). This gives the opportunity to determine the primary sex ratio, by staining the chromosomes of the embryos and inspecting them for the condensed paternal chromosomes (indicating that the embryo is male).

Culture conditions

The culture used for this experiment was obtained from a laboratory culture at Wye College, U.K. All mealybugs were cultured on sprouting potatoes (cultivar Desiree) in rectangular plastic boxes (3.2 litres in volume) covered with fine mesh. Cultures were kept at 25°C, 70% RH and under a photoperiod of 12:12 (L:D) h. These same conditions were also used during both experiments.

Experimental setup

The setup for both experiments consisted of two small plastic rectangular boxes connected by a plastic tube with a diameter of 1cm (Fig. 1), to allow for dispersal between them. In both experiments one of the 2 boxes was randomly assigned the source box and this was where the egg masses were placed to initiate the experiments. In order to obtain individuals of known and equal age for each experiment, 20 ovipositing females were removed from the mass culture and placed on fresh potatoes where they were allowed to oviposit for 24 h. Following oviposition, offspring were allowed to develop for 30 days, by which time they will have reached reproductive maturity and been mated. Females were then isolated from the main culture and placed on a single potato sprout in individual glass tubes and checked daily for signs of oviposition. As it is difficult to handle individual mealybug eggs, density in our experiments was manipulated by varying the number of resulting egg masses from
these females to initiate each replicate. From previous experiments we know that female age strongly influences clutch sex ratio (Ross et al. 2010). This knowledge can be used to manipulate the population sex ratio, by using egg masses of females from different ages. We used eggs from females on the first and sixth day of oviposition. A previous study had shown that the average sex ratio (proportion males in a brood) produced by the females on the first day is 0.81 and the sex ratio of the sixth day is 0.34, while the average clutch sizes are similar (Ross et al. 2010). For the male-biased treatment, we therefore isolated egg masses from females on their first day of oviposition, while for the female-biased treatments we used egg masses produced on the sixth day (these females will be referred to as one day old and six days old).

**Density and sex ratio**

For the first experiment, a single potato was placed in each of the 2 chambers of the dispersal box. The potatoes were weighed at the start of the experiment and matched in size so that the difference in weight between the two potatoes in the chambers was less than 10 g. Depending on the treatment either 10 or 20 egg masses from either one or six day old females were placed on the potatoes in the source box, resulting in four treatments: female biased low density, female biased high density, male biased low density and male biased high density. The eggs were then allowed to hatch and the nymphs to develop and migrate between the boxes. Fifteen days after the boxes had been founded, the dispersal tube was blocked to avoid dispersal of later instars and, after twenty days, the number of males and females was determined based on their morphological differences. The total sample size was 27 across the four treatment combinations.

**Starvation and sex ratio**

In the second experiment, we tested for sex differences in dispersal success when no local food source is available, by comparing the sex ratio of crawlers that migrated successfully to a new food source with those in the control that did not have to migrate to find food. The experiment consisted of one dispersal treatment and two control treatments: a secondary sex ratio control (de-
determined at the third instar) and a primary sex ratio control (determined at the embryo stage). For each of these treatments there are two sex ratio conditions (male biased and female biased, as described previously). For the dispersal treatment, the same setup was used as for the first experiment, although in this experiment a potato was placed only in the sink chamber, while the source chamber remained empty. Depending on the sex ratio treatment, a single egg mass of either a one day or six day old mother was placed in the empty source chamber. For the secondary sex-ratio control, a potato was placed in a single box, without a dispersal tube and the egg mass was placed directly on the potato; for the primary sex ratio control treatment the eggs were taken directly from the ovipositing female (either one or 6 days old) and transferred to fixative and subsequently stained, sexed and counted (Ross et al. 2010). The sex ratio of the secondary sex ratio control and the dispersal treatments were counted 20 days after the populations were founded, again based on morphological differences between the sexes. The total sample size (number of replicates) of the experiment was 50.

Data analysis
All data were analyzed using R (R Development Core Team 2008). All sex ratio data were analyzed using a generalized linear model approach with a quasibinomial error structure to correct for overdispersion. All other data were analyzed using a general linear model with a Gaussian error structure. For the first experiment, we calculated the dispersal probabilities of males and females by dividing the number of successful dispersers of a certain sex by the total number of that sex (summed over the two boxes). For the second experiment, we fitted a generalized linear model to test for the treatment effects on sex ratio. We assumed that, if there was a general mortality difference between the sexes, we would expect to see a difference between the primary SR control treatment and the other two, while if the mortality difference was mainly associated with different success rates of dispersal, then the two controls (primary and secondary sex ratio) should be similar, but the sex ratio in the dispersal treatment different.

Results

Density and sex ratio: Total sex ratio
To manipulate the sex ratio of the source populations, egg masses of females of two different ages were used to setup the source population. This manipulation was successful, as there was a significant effect of the age of the mothers used on the population sex ratio ($F_{1,20} = 126.4; P < 0.001; \text{Fig. 2}$). The boxes founded by females on their first day of oviposition had an average sex ratio of 0.83, whilst the boxes founded by females on their sixth day of oviposition had an average sex ratio of 0.42. To manipulate the density of the populations, boxes were founded with either 10 or 20 egg masses. The total population size in the low-density boxes was on average $209 \pm 36$ and in the high-density boxes $491 \pm 61$. The density treatment did not by itself affect the sex ratio of the population ($F_{1,21} = 0.88; P = 0.36$). However there was a significant interaction between density and the mother’s age (sex ratio treatment) on sex ratio ($F_{1,19} = 6.77; P = 0.018; \text{Fig. 2}$). As such, it seems that, under high density and a male-biased sex ratio, female nymphs have a relatively higher mortality, while at a slightly female-biased sex ratio, higher density leads to relatively higher mortality for male nymphs. However without knowing the primary sex ratio of the treatments, it is hard to confirm that the observed results are the result of differential mortality.
Density and sex ratio: Dispersal

The number of individuals that dispersed to the second box was very low for all treatments with an overall average of 4.22 ± 1.07 individuals moving between boxes and an average dispersal probability of 0.013. There was no effect of either the density of the population, the age of the founding females or the weight of the potato in the source box on the total dispersal rates (age: $F_{1,20} = 0.07$, $P = 0.79$; density: $F_{1,21} = 0.37$, $P = 0.55$; potato: $F_{1,19} = 0.88$, $P = 0.36$) and nor were there any significant interactions between those factors.

In order to test for a difference between the dispersal rates of the sexes and the possible effect of density and sex ratio on this difference, we compared the sex ratio between the source and sink boxes. We fitted the sex ratio in the sink population as response variable, with density and the age of the mother as explanatory variables, and the sex ratio in the source population as covariate. The only factor with a significant effect on the sink sex ratio was the sex ratio in the source box ($F_{1,14} = 18.94$; $P = 0.0009$) and the correlation between source and sink sex

FIG. 2. Results from the first experiment showing: (a) The average sex ratio (error bars showing binomial standard errors) across both source and sink box and (b) the total number of surviving individuals summed over the source and sink boxes (error bars showing standard errors) for the two different density treatments and for mothers of two different ages (day 1 and 6). Sex ratios and number of offspring were counted at the age of 20 days, after individuals were allowed to disperse.

FIG. 3. Dispersal probability of male and female crawlers for each of the four treatment combinations in experiment 1 (LD: low density, HD: high density). The error bars show the binomial standard errors.
ratios did not differ from unity (1.12±0.51). This showed that there was no significant difference between the dispersal rates of the sexes and neither density nor the sex ratio treatment significantly affected the sex ratio of dispersers (density: $F_{1,13} = 0.13$, $P = 0.725$; sex ratio treatment: $F_{1,12} = 0.18$, $P = 0.679$).

**Starvation and sex ratio**

The number of surviving offspring in the dispersal treatment was lower than the number in the two controls ($F_{1,47} = 5.38$; $P = 0.008$; Fig. 4b), suggesting that only a proportion of the crawlers originally present were able to successfully migrate and find the food source (no surviving crawlers were present in the source box). However, the sex ratio treatment did not affect the number of successful dispersers ($F_{1,46} = 0.16$; $P = 0.69$).

In order to compare whether there was a difference in mortality between the sexes and how much of the mortality was caused by a failure to disperse to the new food source, we compared the sex ratio between the two controls and the dispersal treatment. There was a significant difference in sex ratio between the female- and male-biased sex-ratio treatments as expected ($F_{1,45} = 69.65$; $P < 0.001$). However, there were no significant differences among the three treatments (dispersal and the two controls: $F_{1,46} = 1.93$; $P = 0.157$) and additionally, no interaction between the sex-ratio treatments and the three dispersal treatments ($F_{1,43} = 1.31$; $P = 0.28$). Since within each of the two sex-ratio manipulations (male- and female-biased clutches) there was no difference in the sex-ratio between control clutches sexed as embryos (primary sex ratio control), control clutches sexed as adults (secondary sex ratio control) and the actual dispersal treatment, dispersal success does not differ between the sexes and we can also be confident that differential male and female mortality is not confounding these results.

**FIG. 4.** (a) The average sex ratio of surviving offspring (error bars showing binomial standard errors) and (b) the total number of surviving individuals (error bars showing standard errors) for the two controls (primary and secondary sex ratio) and the dispersal treatment for mothers of two different ages (day 1 and 6) for experiment 2.
Discussion

Neither of the two experiments revealed a difference between the sexes in either the dispersal rates or dispersal success, although it must be noted that the number of dispersers in both experiments were very small. The first experiment showed some suggestion that there might be a mortality difference between the sexes, with males having a slightly increased mortality rate. However, as our experiment was not specifically designed to test for differences in mortality, alternative explanations cannot be excluded. Our second experiment, on the other hand, suggests that differential mortality is unlikely to be a major confounding variable.

Based both on behavioural and life-history differences between the sexes, we expected that female crawlers would have a higher dispersal rate than males. This pattern has earlier been observed in a few scale insect species that have sexually dimorphic crawlers: First of all, a few species of armoured scale insects (Coccoidea: Diaspididae) have crawlers that are sexually dimorphic in colour. In these species, it has been observed that female crawlers are more likely to disperse away from the place where they were born than their brothers (Brown and Bennett 1957, Gullan and Kosztarab 1997). Secondly, sex-specific dispersal has been observed in Dactylopius australinus. In this species, female crawlers develop long wax threads, which aid dispersal, while this adaptation is absent in male crawlers (Moran et al. 1982, Greathead 1997). Sex-specific crawler dispersal has also been observed in two species of gall-forming Eriococcids. In these species, males are found to induce a gall on the gall of their mother or to develop within their mother’s gall and therefore generally stay close to the place there were born, while female crawlers disperse and form galls independently (Cook et al. 2000). Additionally, a remarkable form of sex-specific dispersal strategy has also been observed in another Eriococcid genus (Cystococcus), whereby female crawlers are carried to new feeding sites by their adult (winged) brothers (Gullan and Cockburn 1986). All these observations suggest that female crawlers are more likely to disperse than males.

However, no difference was observed between the sexes, although the sample size used was relatively small and the low dispersal rate observed reduced the power of the experiment. One possible explanation for the contrast between our results and earlier observations is that strong differences in crawler dispersal rates between the sexes are only present in species with sexual dimorphic crawlers. Another aspect that could have affected the results of our experiments is that we only considered dispersal distance while there might be sex differences in dispersal behaviour that do not necessarily affect the total distance crawler travel but does affect the distribution of male and female crawlers on the host plant. For example, in many armoured scale insects, it has been shown that male and female crawlers might differ in how they are attracted to light and in which areas of the host plant they prefer to settle (Beardsley and Gonzalez 1975). It has also been shown that in the armoured scale insect Pseudaulacaspis pentagona (Targioni Tozetti), male crawlers congregate in clusters while female crawlers settle more scattered across the host plant (Beardsley and Gonzalez 1975). This suggests that, if the above observations apply to P. citri, there could also be more subtle behavioural differences between male and female crawlers that our experiment is unable to reveal. Finally, we would like to point out that in the experiments presented in this paper we only examined dispersal behaviour via walking, while other forms of dispersal, for example wind dispersal, might also be important and potentially more prone to sex differences.

The overall dispersal rate in the first experiment was very low with no dispersal
events in 30% of the 27 boxes. However, the relative higher numbers of dispersers observed in the second experiment using the same setup did show that crawlers are able to migrate between the boxes. The difference between the experiments, in which a food resource either was or was not present in the source box, suggests that, even under relatively high densities, crawlers tend to settle nearby, rather than trying to find a less crowded spot. Moreover, the fact that density did not have any effect on the total dispersal rate might suggest that the crowding in the high density treatment was insufficient to induce enough competition. Judging what constitutes biologically meaningful levels of density however is difficult (Ross et al. 2010), as estimates of natural density levels are rare (but see Nestel et al. 1995). Other studies considering dispersal behaviour of scale insects crawlers have shown a range of factors influencing dispersal. In the armoured scale insect *Aonidiella aurantii* (Maskell) crawlers start wandering shortly after emergence but the wandering time is affected by host-plant species and quality, with longer wandering times on lower quality hosts (Willard 1973).

To our knowledge, this paper presents the first experimental attempt to test for a potential difference in dispersal behaviour between male and female crawlers in scale insects. The results presented suggest that there is no substantial difference, neither in the dispersal probability nor its success. However, these experimental results need validation under more natural situations. In order to test sex specific dispersal under field conditions, it might be possible to use the cytological techniques described in this paper to sex crawlers found at varying distances from a known source populations. In addition, population genetic techniques, at a sufficient degree of resolution, may also shed light on dispersal patterns. One could compare nuclear and mitochondrial (or endosymbiont) sequences to compare the geographic structuring of female lineages across a population, assuming sufficient variation is present (and it may well not be). Unfortunately population genetic studies on scale insects are rare (but see Cook and Rowell 2007) and to-date, no study has attempted to estimate sex-specific dispersal rates in scale insects using these techniques, and it will no doubt be difficult to get sufficient material to provide a fine-scale of resolution. Additionally although these techniques can shed light on overall differences in dispersal rates between the sexes, they will not be able to reveal at which developmental stage the difference in dispersal rate is most pronounced. Progress will probably be most rapid by exploring the diversity of dispersal strategies observed in nature, in particular in exploring the extent to which crawlers actively or passively disperse, which might then allow more sophisticated experimental manipulations to be undertaken.

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Επίδραση του φύλου στη συμπεριφορά διασποράς των ερπουσών προνυμφών του Planococcus citri

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ΠΕΡΙΛΗΨΗ

Όταν η διασπορά σχετίζεται με το φύλο είναι δυνατό να έχει σημαντικές εξελικτικές και οικολογικές επιπτώσεις, επηρεάζοντας τη δομή των πληθυσμών, την αναλογία φύλου καθώς και την ταχύτητα με την οποία αποκινούνται νέα ενδιαιτήματα. Στα κοκκοειδή έντομα, οι προνύμφες πρώτης ηλικίας (έρπουσες) θεωρούνται ως το κύριο στάδιο διασποράς. Αν και στα κοκκοειδή έντομα υπάρχει μορφολογικός διμορφισμός φύλου, στα περισσότερα είναι αδύνατος ο διαχωρισμός φύλου στο στάδιο των ερπουσών προνυμφών. Στην παρούσα έργασία μελετήθηκε η διασπορά του εντόμου Planococcus citri (Risso) (Hemiptera: Coccidae) από και προς διαθέσιμα ενδιαιτήματα. Σκοπός της μελέτης ήταν να εξετάσουμε αν: (1) η συμπεριφορά διασποράς στις έρπουσες προνύμφες διαφέρει στα δύο φύλα και πώς επηρεάζεται από συνθήκες όπως η πυκνότητα πληθυσμού ή η αναλογία φύλου, και (2) αν τα δύο φύλα διαφέρουν ως προς την επιτυχία διασποράς σε νέο φυτό ξενιστή. Σύμφωνα με τα αποτελέσματα μας παρατηρήθηκε ότι τα αρσενικά και θηλυκά άτομα κατά το στάδιο της έρπουσας προνύμφης δε διαφέρουν στη συμπεριφορά διασποράς όταν αυτή συμβαίνει με βαδιστική μετακίνηση. Αυτά τα εργαστηριακά πειράματα αποτελούν ένα σημαντικό πρώτο βήμα για την κατανόηση της εξέλιξης της συμπεριφοράς διασποράς του P. citri στη φύση ενώ απαιτούνται περαιτέρω πειράματα με διαφορετικούς τρόπους διασποράς, εκτός της μετακίνησης, για να κατανοήσουμε καλύτερα τη δεδομένη συμπεριφορά του εντόμου.