Animal Behaviour 132 (2017) 247–252

Contents lists available at ScienceDirect

Animal Behaviour

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

Parasite dispersal risk tolerance is mediated by its reproductive value

Maxcy P. Nolan IV, Keith S. Delaplane*

Department of Entomology, University of Georgia, Athens, GA, U.S.A.

ARTICLE INFO

Article history:
Received 14 November 2016
Initial acceptance 11 January 2017
Final acceptance 21 June 2017

MS. number: A16-00997R

Keywords:
Apis mellifera
dispersal evolution
epidemiology
honeybee
host choice
Varroa destructor
varroa mite

Parasite dispersal theory draws heavily upon epidemiological SIR models in which host status (susceptible (S), infected (I), or recovered (R)) is used to study parasite dispersal evolution. In contrast to these extrinsically host-centric drivers, in this study we focus on an intrinsic driver, the parasite's reproductive value (predicted future offspring) as a regulator of the extent to which the individual will engage in risky dispersal behaviour. As a model system we use the honeybee *Apis mellifera* and its ectoparasite, the mite *Varroa destructor*. Mite reproduction happens exclusively inside cells of bee brood, and newly emerged fecund mites may parasitize either a homocolonial brood cell (low risk dispersal) or emigrate to a new bee colony via phoretic attachment to mature forager bees (high risk dispersal). In an empirical bioassay, prepartum mites (high reproductive value) and postpartum mites (low reproductive value) were offered a choice of newly emerged homocolonial worker bees (low risk), homocolonial pollen forager bees (high risk), or heterocolonial pollen foragers (high risk). A preference for newly emerged bees was earlier and more strongly sustained among prepartum mites. This suggests comparatively greater dispersal risk tolerance among postpartum mites with lower reproductive value. A dangerous bid for dispersal may be adaptive if the individual has already successfully reproduced and the rewards for successful dispersal are sufficiently large.

The choice between staying at a proven resource and dispersing to a new one is a high stakes decision for all parasites. Modern strategies for dispersal can be viewed as evolutionary optima balancing its benefits, such as improving conditions for reproduction (Ruxton & Rohani, 1999), avoiding kin competition (Cote & Clobert, 2010) and avoiding inbreeding (Crespi & Taylor, 1990), against its risks, such as energetic costs (Stirling, Fairbairn, Jensen, & Roff, 2001) and direct mortality (Bowler & Benton, 2009). Moreover, it is increasingly understood that dispersal is not a simple diffusion event, but rather a product of interacting dynamics at population margins.

Dispersal at the level of parasites is often studied with epidemiological 'SIR' models, which categorize hosts as susceptible (S), infected (I) or recovered (R) (Anderson & May, 1979, 1982). Parasite transmission from I to S hosts is regulated by a few powerful drivers, including host density (Kermack & McKendrick, 1927; Peel et al., 2014), host genetic diversity (Lively, 2010), genetic relatedness of other parasites on near-neighbour hosts (Lion & Boots, 2010) and relative opportunities for local versus global transmission (Boots & Sasaki, 1999). Such host-centric SIR models have been successfully used to explain disease dynamics in systems as diverse as rabies in fox (Anderson, Jackson, May, & Smith, 1981) and Ebola in humans (Pandey et al., 2014).

In the case of a relatively long-lived parasite capable of multiple broods, it is likely that dispersal regulation includes drivers intrinsic to parasite state. One of these is kin structure of parasites at the population level (Cote, Clobert, & Fitze, 2007; Hamilton & May, 1977; Kubisch, Fronhofer, Poethke, & Hovestadt, 2013). Theory predicts that dispersal will be selected for if inclusive fitness gains (reduced competition) exceed dispersal costs for the emigrant (Hamilton & May, 1977).

Another likely intrinsic dispersal driver is reproductive value at the individual level. An individual's reproductive value, defined as predicted future reproductive success based on the individual's age and sex (Fisher, 1930; Williams, 1966), has been used in applications as diverse as parental investment theory (Albrecht & Klvaňa, 2004; Ghalambor & Martin, 2001; Redondo & Carranza, 1989), mate selection (Wolf & Schulman, 1984), evolution of senescence (Hamilton, 1966) and for predicting success of colonists (MacArthur & Wilson, 1967).

In this study, we investigate an association between a parasite's reproductive value and its propensity to engage in risky dispersal

* Correspondence: K. S. Delaplane, Department of Entomology, University of Georgia, Athens, GA 30602, U.S.A.
E-mail address: ksd@uga.edu (K. S. Delaplane).

http://dx.doi.org/10.1016/j.anbehav.2017.08.016

behaviour. We use the western honeybee *Apis mellifera* and its ectoparasite, the mite *Varroa destructor*, as a model system. This host–parasite relationship is man-assisted and no older than the mid-19th century (Danka, Rinderer, Kuznetsov, & Delatte, 1995). However, each species now shows signs of co-adaptation (Fries & Bommarco, 2007; Seeley, 2007); indeed the taxonomic epithet *destructor* is contextual to *A. mellifera* (Anderson & Trueman, 2000). The mite reproduces exclusively inside cells of bee brood. Emerged fecund females may either parasitize a new cell of brood in the same colony, often aided by brief phoretic attachment to a young house bee, or emigrate to a new bee colony and access a new population of host brood. This emigration is accomplished by phoretic attachment to adult forager bees who may subsequently enter an alien bee colony. This bee behaviour, called drifting, can be understood as either a bid by the drifter for reproductive opportunity (Neumann, Radloff, Pirk, & Hepburn, 2003) or a simple accident of navigation (Free, 1958). But from the perspective of the obligate mite parasite, a decision for extracolonial dispersal certainly constitutes the riskier choice.

Our study of the regulatory effect of mite reproductive value on dispersal risk tolerance was accomplished with laboratory behavioural assays comparing high- and low-risk host choices in pre- and postpartum varroa mites. Because most female varroa mites complete fewer than two reproductive cycles (DeRuijter & Calis, 1988; Fries, Camazine, & Sneyd, 1994), prepartum mites have a higher reproductive value. We predicted that this cohort would exhibit lower risk tolerance by preferentially parasitizing low-risk hosts, in our case, young homocolonial bees. In contrast, we predicted that postpartum mites would exhibit higher risk tolerance and parasitize older forager bees, either homocolonial or heterocolonial, at a comparatively higher rate.

METHODS

Collecting and Marking Mites

We collected mites from infested bee colonies maintained by the University of Georgia using one of two methods. The first method involved the use of a bee repellent (Bee Go, Cloverland Products, Inc., Pearl City, IL, U.S.A.) to drive adult bees into a box measuring 46.4 × 41.3 × 30.5 cm (Aliano & Ellis, 2005). The bees were then dusted with powdered sugar inside the box to dislodge and capture mites. The second method involved dusting the tops of all frames with powdered sugar and collecting mites as they fell through screen bottom boards onto plastic boards placed under the colony. Living mites were brought into the laboratory and housed on water-moistened filter paper suspended inside a clean glass quart (~1 litre) jar. Jars with mites were maintained in an incubator at 32 °C and ~40% relative humidity while marking was completed. Marking was accomplished the same day mites were collected. All mites were marked with correction fluid using the protocol described in Kirrane et al. (2012). After marking, mites were inoculated into brood cells containing 10-day-old honeybee larvae (see below).

Preparing Honeybee Worker Larvae for Inoculation

Ten days prior to mite inoculation, queens from four test colonies were individually caged on an empty drawn deep comb for 24 h to ensure uniform age of developing larvae. Queens were moved to a new frame every 24 h; each frame was labelled with the date eggs were laid. This was done for four consecutive days. Frames with 10-day-old larvae were removed from their colonies; adult bees were brushed off in the field before frames were brought back to the laboratory for inoculation. A scalpel was used to make a slit in the capping, a marked mite was placed inside the cell, and the slit was gently pushed back into place. A sheet of transparency film was used to map the inoculated larvae to aid in mite recovery. Frames were returned to the parent colony immediately after mite inoculation. This procedure was performed for each of the four test colonies. Inoculations continued for 4 days and were performed in a darkened room at 32 °C at ~40% relative humidity to improve survivorship of mites and honeybee larvae.

Mites were recollected 10 days after inoculation when bee larvae were 20 days old. Frames were removed, adult bees brushed off and the frames brought back to the laboratory. With the aid of the mapped transparency films, cells of inoculated brood, now pupae, were manually uncapped with forceps. All cells containing marked mites and their offspring were collected for experimental trials.

Only marked postpartum mites and their unmarked prepartum daughters were used in the study. Unmarked prepartum mites are assumed to have never reproduced while marked postpartum mites are assumed to have reproduced at least once. Only mites originating from a cell with a marked mite were used in the study. To control for honeybee larvae that may have already contained a postpartum mite in addition to the marked inoculated mite, each mite was inspected carefully, and the marked mite as well as any mites that were obviously lightly sclerotized, were used. No unmarked, darkly sclerotized mites were mistakenly used as prepartum mites.

The two cohorts of mites were placed on water-moistened filter paper suspended inside pint-sized (~0.5 litre) glass jars and placed in an incubator at 32 °C and ~40% relative humidity. Mites were used in trials the day of collection.

Collecting Worker Honeybees

The same four colonies that produced pre- and postpartum mites were used as source colonies for adult honeybees. Mite-free, newly emerged teneral workers (NEW) found when searching for marked mites were used as NEW bees. Pollen foragers were collected directly off the comb; only bees with pollen in their corbicula were used.

Each of the four test colonies was positioned at least 3.2 km from each other or any other known colony to minimize the chance of bees drifting between the four colonies.

Bees were housed in new Ziplock® plastic food boxes with air holes and provided 1:1 sugar water. They were held in an incubator at 32 °C and ~40% relative humidity until used. Bees were used within 24 h of collection. Bees were immobilized with CO_2 for placement into petri dishes and examined for phoretic mites as they were being added. Bees found with phoretic mites were not used.

Mite Trials

Mite choice trials were conducted over four consecutive days utilizing a different mite source colony each day. Each petri dish trial consisted of a Fisherbrand® 100 × 15 mm petri dish containing one mite and three bees. The mite was given a choice of three living bees: (1) a homocolonial NEW bee, (2) a homocolonial pollen forager and (3) a heterocolonial pollen forager from one of the other three test colonies. Each combination was replicated three times for each mite type (Table 1).

Bees were immobilized with CO_2, inspected for phoretic mites, and once deemed mite free, placed equidistant from each other around the sides of the petri dish. Bees had either their right, left, or both forewings clipped for cohort identification. Clipping occurred just prior to being placed in the petri dish. Petri dishes were placed