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Caste-specific differences in risk sensitivity in honeybees, *Apis mellifera*

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Honeybee workers (foragers) are risk averse to variability in volume of reward when measured by conditioning of the proboscis extension response, and the level of risk aversion depends on the coefficient of variation of the variable distribution. Since drones do not forage on flowers, they may not have been under selection for risk-sensitive choice behaviour. We compared risk sensitivity of workers and drones and their ability to discriminate between the reward volumes used in the risk sensitivity experiments. Both castes discriminated better between 0 and 0.4 μl than between 0.4 and 1.2 μl, consistent with Weber's law of relative discrimination. Workers discriminated between both volume pairs better than drones, and workers showed greater risk aversion than drones. This is the first demonstration of caste-specific differences in risk sensitivity. These differences do not appear to be the result of differences in energy budgets, since both castes were on positive energy budgets. Levels of risk aversion were consistent with the coefficient of variation model. We calculated the relative associative strengths of subjects to the reward volumes from their choice proportions in the discrimination tests. The relative associative strengths of workers were greater than those of drones, and in both castes the relative associative strength of 0.4 μl relative to 0 μl was greater than that of 1.2 μl relative to 0.4 μl. Owing to Jensen's inequality, the decreasing functions of differences in relative associative strengths could explain differences in degree of risk aversion between the castes. Our findings are consistent with both mechanistic and functional explanations.

Risk sensitivity refers to how an animal responds to variability in resource distribution. Risk-sensitive foraging behaviour has been modelled from both a mechanistic (proximate) and a functional (ultimate) perspective. Mechanistic models have incorporated learning theory (Montague et al. 1995; Shapiro 2000; Shapiro et al. 2001), memory constraints (Real et al. 1990; Real 1996) and perceptual biases in memory and evaluation (Reboreda & Kacelnik 1991; Kacelnik & Bateson 1996; Perez & Waddington 1996; Shafir 2000; Waddington 2001). Functional models have incorporated thresholds for survival and reproduction (Stephens & Krebs 1986; McNamara et al. 1991; Hurly 2003). The mechanistic and functional approaches are complementary, and together contribute to a better understanding of risk-sensitive foraging behaviour (Kacelnik & Bateson 1996).

Risk sensitivity is often interpreted within two (not mutually exclusive) general frameworks: Jensen's inequality and Weber's law. Jensen's inequality results from nonlinear utility functions (Smallwood 1996). These nonlinearities may originate in the perception and processing of information, or may describe fitness consequences. The degree of risk sensitivity depends on the deviation of these functions from linearity, with concave-down functions resulting in risk aversion, and concave-up functions resulting in risk proneness. Weber's law relates the perceived difference between stimuli to the mean value of the stimuli; it may manifest itself during stimulus evaluation or memory retrieval (Perez & Waddington 1996; Kacelnik & Abreu 1998).

Nectarivores often forage in multispecies floral patches, each plant species characterized by a distribution of nectar rewards (Real & Rathcke 1988; Petanidou & Smets 1995; Shafir et al. 2003). Thus, nectarivores have received much attention in risk sensitivity studies. Honeybees, in particular, have been considered risk averse in some cases (Shafir

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et al. 1999; Shapiro 2000; Shapiro et al. 2001) and risk indifferent in others (Banschbach & Waddington 1994; Fulop & Menzel 2000). Shafir (2000) stressed the fact that levels of risk-sensitive choice behaviour form a continuum, and hence we should measure the degree of risk sensitivity, rather than distinguish between risk sensitivity and risk indifference. Furthermore, levels of risk sensitivity are affected by characteristics of the reward distribution, such as the coefficient of variation, and possibly the skew of the distribution (Shafir 2000; Shafir et al. 2003). Differences in experimental design and reward distributions are probably the reason for differences in risk sensitivity between studies with the same species.

Shafir et al. (1999) modified the conditioning of the proboscis extension response (PER) paradigm for the study of risk sensitivity in honeybees. The main advantage of this system is that it permits control of variables that are critical to studies of conditioning (Rescorla 1988). In particular, the presentation of conditioned stimuli is precisely regulated in comparison to free-flying conditions in which risk sensitivity in bees is usually studied (Real 1981; Waddington et al. 1981; Waddington 1995; Fulop & Menzel 2000; Shapiro 2000; Shapiro et al. 2001). Another advantage, which we exploit here, is that it allows comparisons between drones and workers (Benatar et al. 1995; Pankiw & Page 1999; Chandra et al. 2000).

Honeybee drones are fed by workers in the colony, or feed themselves from the colony's honey stores, which provides a relatively constant high-quality food supply (Winston 1987). Hence, from an evolutionary perspective, drones need not have evolved the cognitive architecture (Real 1991) to deal with variable resources, and the survival and reproduction thresholds of functional models do not apply to them. We would therefore expect them to be risk indifferent. Workers, on the other hand, should have evolved risk-averse behaviour, at least when on a positive energy budget (Stephens & Krebs 1986).

From a mechanistic perspective, levels of risk sensitivity of workers and drones should reflect the shape of their respective evaluation functions of different nectar amounts. In a discrimination procedure, we tested how well workers and drones discriminated between various reward amounts. We then tested the two castes in risk sensitivity experiments that incorporated the same reward amounts. We also assessed whether potential differences in energy budgets between workers and drones could account for differences in risk sensitivity.

METHODS

Restraint of Subjects

Bees were maintained in standard honeybee hives at the apiary of the B. Triwaks Bee Research Center, Rehovot, Israel, and were free to fly and forage. We harnessed subjects as in Shafir et al. (1999). Workers were collected into small glass vials as they exited the hive. Drones were collected from a drone trap at the entrance of the hive. Such a trap has a grid that is wide enough for workers to pass through but too narrow for drones to pass. Thus, drones are trapped as they try to exit the hive. To facilitate harnessing of the workers, each vial was submerged into ice water until the bee stopped moving. This was not necessary for the drones, which cannot sting. Subjects were then strapped into a sectioned hollow plastic tube by a 3-mm-wide strip of duct tape that wrapped around the tube and (dorsal) thorax of the bee. The abdomen of the bee was not covered. Subjects were harnessed so that the stand extended to just below the front pair of legs, which were loose over the stand, to ensure that the head of each bee was free to rotate. Bees that were captured and harnessed were killed at the end of the experiment by placing them in a freezer. We did not release bees from the harnesses, since the procedure of removing the duct tape from the bees could be stressful for the bees, and some bees could be injured by the procedure. Furthermore, bees returning to the hive with foreign scents on their bodies might not be accepted by the guard bees at the entrance, or suffer from aggressive interactions from nestmates within the hive. The small number of bees captured relative to the number of foragers in a honeybee colony was negligible, so the colony did not suffer any adverse effects from the missing captured bees.

In discrimination experiment 1, in which only workers were tested, subjects were collected in the morning, fed 0.6 μl of 1.5-M sucrose solution 40 min after being harnessed, and allowed to acclimate for 2 h. The antennae of each subject were then touched with a drop of sucrose solution, and only those that extended their proboscides were selected; typically very few bees do not pass this performance criterion. In the other experiments, which included drones, subjects (workers and drones) were collected in the early afternoon, when drones exit the hive for mating flights. After harnessing, subjects were fed 20 μl of sucrose solution to allow them to survive the night. Drones tend not to imbibe such large amounts at once, so they were fed smaller amounts every 30 min for up to 2 h. Subjects were left overnight in a dark room at 25 °C. The next morning (after a 14–16-h wait) they were fed 1 μl, and we chose those that extended their proboscides and fed.

Apparatus

Odours were delivered to subjects from two 1-ml glass syringes mounted at a training station. We used geraniol and 1-hexanol as odours because honeybees discriminate well between these odours in proboscis extension learning experiments (Smith 1997; Shafir et al. 1999). We added 3 μl of pure odour to a strip of filter paper, which was placed inside a syringe. The tip of each syringe was attached by silicone tubing to a valve that was attached to an air pump. Valves were controlled by a computer and opening of a valve caused an odour air stream flow of 5 cm^3/s out of the tip of the syringe.

In choice trials, we attached each syringe to a base that mounted on to tracks at the training station. Syringes were mounted horizontally so that when we placed a subject in the training station the tips of the syringes were 12 mm from the bee and pointed towards the base of the bee's