

Intercolony Variation in Learning Performance of a Wild British Bumblebee Population (Hymenoptera: Apidae: *Bombus terrestris audax*)

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The first quantitative assessment of between-colony variation in learning ability within a natural bee population is presented here. Bumblebee (*Bombus terrestris audax* Harris 1776) colonies were raised in the laboratory, under identical conditions, from wild caught queens. 240 bumblebee workers from 16 colonies were individually tested in an ecologically relevant foraging situation in which they had to distinguish yellow, rewarding artificial flowers from blue, unrewarding ones under laboratory conditions. During the initial stages of the task, 15 colonies showed a very strong, unlearned preference for blue flowers (the other colony showed no strong colour preference). There was significant variation among the colonies tested in learning speed, task saturation performance, and the number of flower choices made prior to first feeding from a rewarding, yellow flower. Such intercolony variation in performance forms the raw material upon which any selection for learning ability might act. Overall, neither age nor size of bees were consistently correlated with learning performance, but older bees learned faster in one of the colonies, an effect that remained significant even after statistical correction for multiple comparisons.

Key words: *Bombus terrestris audax* Harris 1776 – associative learning – flower colour – foraging – learning speed – learning curve – nectar

RAINE N E, INGS T C, RAMOS-RODRIGUEZ O & CHITTKA L [Biol & Chem Sci, Queen Mary, London, E1 4NS]: **Unterschiede im Lernverhalten zwischen Kolonien einer freilebenden Britischen Hummelpopulation (Hymenoptera: Apidae: *Bombus terrestris audax*)**. – Entomol Gener 28(4): 241–256; Stuttgart 2006-05. [Abhandlung]

Die Unterschiede in der Lernfähigkeit zwischen einzelnen Kolonien einer natürlichen Hummelpopulation (*Bombus terrestris audax* Harris 1776) wurden untersucht. Die Kolonien wurden von in freier Natur gefangenen Königinnen unter identischen Bedingungen im Labor aufgezogen. 240 Hummelarbeiterinnen aus 16 Kolonien wurden im Labor in einer ökologisch relevanten Fouragier-Aufgabe getestet, in welcher sie gelbe, belohnende Kunst-Blüten von blauen, unbelohnenden Blüten unterscheiden mußten. Die Kolonien zeigten signifikante Unterschiede in mehreren Aspekten ihres Verhaltens bei dieser einfachen assoziativen Lernaufgabe. Anfänglich zeigte sich bei 15 der getesteten Kolonien eine starke, angeborene Präferenz für die Farbe blau (während sich bei der sechzehnten Kolonie keine ausgeprägte Präferenz zeigte). Außerdem manifestierten sich signifikante Unterschiede zwischen Kolonien bezüglich der Lerngeschwindigkeit, der nach Abschluß des Lernens erbrachten Leistung, sowie der Zahl der Blütenbesuche, die vor dem ersten Besuch einer gelben Blüte gemacht wurden.

Solche Unterschiede zwischen Kolonien formen das Rohmaterial, an welchem die natürliche Selektion für Lernfähigkeit bei sozialen Insekten ansetzen kann. Insgesamt korrelierten weder das Alter noch die Körpergröße der Tiere mit ihren Lernleistungen, aber in einer einzelnen Kolonie lernten ältere Individuen schneller, ein Effekt, der auch nach einer Korrektur für multiple Tests signifikant blieb.

Schlüsselbegriffe: *Bombus terrestris audax* Harris 1776 – assoziatives Lernen – Blütenfarbe – Fou-
ragieren – Lerngeschwindigkeit – Lernkurve – Nektar

1 Introduction

In addition to considerations of the mechanisms of bee learning in the laboratory [CHITTKA, THOMSON & WASER 1999, MENZEL 2001, CHITTKA, DYER, BOCK et al 2003, GIURFA 2003], there has been much speculation on how learning is adapted to real ecological conditions. Animal species differ widely in their cognitive capacities, and it seems commonly assumed that such differences reflect adaptations to the natural conditions under which these animals operate [GALLISTEL 1990, DUKAS 1998, SHETTLEWORTH 1998]. The evidence for this view comes mostly from interspecific comparisons and correlative studies [DUKAS & REAL 1991, SHERRY 1998]. A more informative way to address this question might be to examine the link between intraspecific variation in learning ability and fitness under ecologically relevant conditions. A necessary prerequisite to such a study is a detailed assessment of the variation in learning ability which exists within a natural population, as this variation is raw material upon which natural selection can act.

As a first step in working towards such a potential adaptive explanation for learning behaviour, the results of the first such assessment of intraspecific variation in learning ability are presented here. The study population was the British population of a common European bumblebee species *Bombus terrestris* (*B t audax* Harris 1776). Under natural conditions, bees forage in a complex floral market typically containing dozens of flower species, which differ in their nectar and pollen rewards, their handling costs and spatial distribution. Depending on reward production patterns and the activities of other flower-visitors, the average rewards in a flower species may change rapidly during the course of the day [HEINRICH 1979, WILLMER & STONE 2004]. The ability of bees to respond to such a dynamic foraging environment, as indeed they do [CHITTKA 1998, MENZEL 2001], makes them good candidates for variation in learning ability which could be under selective constraints. Certain learning strategies might be particularly advantageous to bees in such a dynamic floral market, either in terms of maximising total energy gain or avoidance of variation in reward quality, potentially causing them to be preferentially selected over evolutionary timescales.

In order to establish if such processes could be happening, it is first necessary to quantify the actual levels and types of variation in learning performance that occur within a natural population. The focus of this study is the learning performance of bees when faced with the ecologically important associative learning task, that of using flower colour as a predictor of floral reward. Bumblebee workers were tested in the laboratory in a simple foraging situation in which they had to distinguish yellow, rewarding artificial flowers from blue, unrewarding ones. This simple learning paradigm allows us to quantify intraspecific variation in many aspects of this learning task, e.g learning speed, which could have significant effects on foraging performance under natural conditions. In most species, variation between individual organisms represents the raw material for evolution. However in social insects, like bumblebees, the unit of selection is the colony. The evolutionary success of the colony is dependent on the success of the foraging workers; thus colonies with more efficient foragers will tend to produce larger numbers of sexual offspring [SCHMID-HEMPEL & SCHMID-HEMPEL 1998, PELLETIER & MCNEIL 2003]. Therefore, in order to quantify the actual variation in learning ability within a natural population, large numbers of individuals from large numbers of colonies were tested.

In addition to colony membership, the bumblebee learning performance of worker bumblebees could potentially be affected by both their age and size. Indeed, worker age has been shown to influence the performance of honeybees (*Apis mellifera* L.) in olfactory learning experiments [RAY & FERNEYHOUGH 1997, LALOI, GALLOIS, ROGER et al 2001], so it could also be an important factor in a visual learning task for bumblebees. In addition, there is a great deal of size variation among bumblebee workers from the same colony, and variation in worker size has been linked to differences in their foraging performance [GOULSON, PEAT, STOUT et al 2002, SPAETHE & WEIDENMÜLLER 2002, SPAETHE & CHITTKA 2003, INGS, SCHIKORA & CHITTKA 2005]. Thus, size variation could also potentially affect learning speed [WORDEN, SKEMP & PAPAJ 2005]. Therefore the potential effects of size and age on bumblebee learning performance were also investigated.

In order to assess the true potential significance of intercolony variation in learning performance within a natural population, this study has two main aims: firstly, to obtain accurate quantitative estimates of the range of variation in learning ability at both the individual bee and colony level. The relative extent of variation within each colony, compared to the variation among colonies, enables us to more accurately assess the potential significance of the intercolony variation in learning performance, which is ultimately the raw material for any evolutionary processes acting on learning. Secondly, to determine how the learning performance of colonies are distributed within the population: is there continuous or discontinuous variation among colonies? What might such differences signify in terms of selection, past or present, on learning performance?

2 Methods and materials

2.1 Collection and rearing

295 bumblebee queens from the British population of *Bombus terrestris* (*B t audax* Harris 1776) were collected from 4 locations across Greater London (Windsor Great Park, Regent's Park, Hyde Park and South Woodford) from 8th February to 22nd April 2004. After screening for digestive tract parasites by microscopic examination of faeces, 208 unparasitised queens were set up in bipartite, wooden nest boxes (28 x 16 x 11cm). Nest boxes were kept in a controlled environment, 28 °C and 65% relative humidity, and fed defrosted pollen (Koppert Biological systems, UK) and artificial nectar (Apiinvert®, E H Thornes, UK) ad libitum. Feeding and other necessary colony manipulation (eg marking workers) was conducted under red light, otherwise nest boxes were kept in unlit conditions. Hence, bees were not exposed to any coloured stimuli associated with food prior to experiments. Raising wild bees in the laboratory under such identical conditions allows us to control for the environmental component of variation in learning ability. This step is crucial because any differences in the environmental conditions, e.g temperature, in which bees from the same colony are raised could differentially affect their learning performance as adults [TAUTZ, MAIER, GROH et al 2003]. Developing colonies were inspected daily and all newly eclosed workers were recorded and marked on the thorax with individually numbered, coloured tags (Opalith tags, Christian Graze KG, Germany), this enabled us to record bee age when assessing its learning performance. The thorax width of tested bees was measured to allow us to control for forager size in our analyses.

2.2 Training bees and the learning task

Bumblebees (*B t audax*) workers were tested individually in a simple foraging situation in which they had to distinguish yellow, rewarding artificial flowers from blue, unrewarding ones. For testing, each laboratory raised bee colony was connected to a wooden flight arena with a transparent, UV-transmittent Plexiglas lid (120 x 100 x 35cm), by means of a colourless Plexiglas tube.

Shutters along the length of this tube allowed the experimenter to control the traffic of bees between the nest box and flight arena. Bees were pre-trained to forage on 20 bicoloured, blue and yellow, artificial flowers placed in the flight arena. The square (24 x 24mm), bicoloured pre-training flowers were constructed from two halves (each 12 x 24mm), one yellow (Perspex® Yellow 260) the other blue (Perspex® Blue 727). During pre-training all flowers were rewarded with a 15 µl droplet of 50% sucrose solution, placed in the middle of the flower. This provided previously colour-naïve bees with an equal chance to associate both these colours with reward during this pre-training period. Results from a pilot study using colourless, rather than blue-yellow bicoloured, artificial flowers during pre-training indicated a high proportion of bees tested would never probe a yellow flower, and that bees which did would make a very large number of flower choices before probing their first yellow flower. Thus, it was decided that using blue-yellow bicoloured flowers during pre-training would enable us to quantify variation in learning performance on an appropriately large scale. Flowers were placed on vertical transparent glass cylinders (diameter = 10mm; height = 40mm) to raise them above the floor of the flight arena. Bees were allowed to forage freely from these bicoloured pre-training flowers, which were replenished as soon as all the reward on any flower had been consumed. The number of foraging trips (bouts) made into the flight arena by each, individually marked, bee were observed to ensure only strongly motivated foragers were selected for further training. Bees had to complete at least five consecutive foraging bouts on the bicoloured flowers before being selected: most bees selected experienced 5–10 bouts, with very few completing more than 20.

Motivated forager bees were trained individually, in a flight arena containing 10 blue and 10 yellow artificial flowers. The yellow flowers each provided 15 µl of 50% sucrose solution reward, whilst the blue flowers were empty (unrewarding). Bees were regarded as choosing a flower when they either approached (inspected), or landed on it. Bees landing on rewarding flowers did not always feed from (probe) them. The choice sequence made by each foraging bee was recorded until it made at least 100 flower choices starting with the first time it fed from (probed) a yellow, rewarding flower. This criterion was chosen as some individuals made a very large number of flower choices (≤ 373 choices) before probing a rewarding flower. The initial spatial arrangement of flowers was randomly allocated within the flight arena. All flowers were changed between subsequent foraging bouts and their spatial positions reshuffled, to eliminate the possibility that a forager could use scent marks, or the position of previously visited flowers as predictors of reward. During training, flowers were the same size, shape and presented in the same way as in pre-training. Hence the only differences were the colours (flowers were either yellow or blue, rather than bicoloured), and the presence (yellow) or absence (blue), rather than ubiquity of reward (bicoloured) in the respective flowers.

This relatively simple learning paradigm was selected because it allowed us to quantify the learning performance of a large number of individual bees from a large number of colonies: 240 workers from 16 colonies. The colours were chosen to be perceived as strongly different by the bee visual system, so that flower discriminability would not be a performance limiting factor. The spectral reflectance of the two artificial flowers colours was quantified, and converted into colour loci in bee colour space [DYER & CHITTKA 2004, CHITTKA & KEVAN 2005]. The distance between yellow and blue flowers is 0.475 colour hexagon units: distances of 0.2 and above are considered very well distinguishable, and larger distances do not produce further performance improvements [CHITTKA & RAINE 2006, DYER 2006]. These flower colours were specifically selected so that bees had to overcome their strong, unlearned preference for blue, before associating one of their innately least favoured colours (yellow) with reward [BRISCOE & CHITTKA 2001, CHITTKA, INGS & RAINE 2004]. Hence, despite the relative simplicity of design, this task was sufficiently difficult to allow us to detect differences in learning performance both between individuals and between colonies. Controlled illumination for all experiments was provided by high frequency fluorescent lighting [(TMS 24F) lamps with HF-B 236 TLD (4.3 Khz) ballasts, Philips, Netherlands fitted with Activa daylight fluorescent tubes, Osram, Germany] which simulates natural daylight above the bee flicker fusion frequency.

2.3 Analysis of learning data

Learning curves were fitted to the flower choice data for each individual bee to capture the dynamic nature of the associative learning process in this task. The flower choices made by each bee were evaluated as the number of errors (choices of unrewarding, blue flowers) made in each group of 10 flower choices, starting with the first feeding event at a yellow flower, until the bee had completed 100 choices. This grouping of flower choices (i.e. 10 bins, each comprised of 10 choices) represented the best compromise between sufficient repeated measurements of task performance to accurately describe dynamic learning whilst maintaining a robust performance estimate within each bin. The starting point for each bee's learning curve was assessed as the number of errors (blue flowers chosen) during the first 10 flower choices made during its first training bout, i.e. before the bee had probed a rewarding, yellow flower for the first time. For bees that made fewer than 10 flower choices before probing a yellow, rewarding flower (44 of 221), the colony mean (calculated from bees making more than 10 such flower choices) number of errors was used. First order exponential decay functions were fitted to these eleven data points for each individual bee, using the following formula in Microcal Origin® [CHITTKA & THOMSON 1997, CHITTKA et al 2004]: $y = y_0 + Ae^{-x/t}$. Here, x is the number of flower choices made by a bee, starting with the first time it probed a yellow, rewarding flower, and y is the number of errors (choices of unrewarding, blue flowers). The saturation performance level (y_0) is the number of errors made by a bee when it finishes the learning process, i.e. when the bee reaches a performance plateau. The decay constant (t) is a measure of learning speed: high values of t correspond to slow learning, whereas lower t values are generated by faster learners (t values are low when the learning curve approaches y_0 rapidly, and high when the slope of the learning curve is shallow). A is the curve amplitude: it specifies the maximum displacement (height) of the curve above y_0 . This is equivalent to the difference in number of errors (performance levels), made at the start and end of the experiment. To ensure realistic learning curves were fitted to the data, the amplitude (A) was constrained within the range 0–10, and it was specified that saturation performance (y_0) should not fall below 0 errors during the fitting process within the Microcal Origin® software. Data were analysed using non-parametric statistics because the distribution of some variables were highly non-normal and resisted transformation (particularly y_0 which is highly right skewed with many 0 values), and also that the number of bees tested in two colonies was limited by their overall colony size (Tab 1).

In order to test for potential age or size effects on learning performance, both of these variables were each correlated (using Spearman's rank correlations) with learning speed (t) and the number of flower choices made by bees prior to probing their first yellow flower. Bonferroni correction was applied to the correlation results from these four tests for each colony ($\alpha = 0.0125$), and the patterns produced by corrected and uncorrected results were compared.

3 Results

3.1 Completion of the learning task

Almost 8% (19 of 240) of bees tested never probed a rewarding, yellow flower (Tab 1), and thus could not be included in further analyses. These bees would persistently continue to choose blue flowers, despite receiving no reward, sometimes foraging in the arena for as long as 20 minutes. Testing was ceased if bees stopped flying for more than a 5 minute period. Bees that never probed a yellow flower were found in 7 different colonies. In the majority of these colonies they were relatively infrequent (6–17%), however they made up almost half of the bees tested in colony A24 (9 out of 21–43%). There were no significant differences in the age (Mann-Whitney $U = 44$, $p = 0.508$) or size ($U = 46$, $p = 0.602$) of the bees from colony A24 which never probed a yellow flower compared to those which did.

Tab 1: The total sampling effort invested in testing the bumblebee learning performance (*Bombus terrestris audax* Harris 1776 – Hymenoptera: Apidae). Tested bees which never probed rewarding, yellow flowers (column 2) could not be included in subsequent analyses. Bees which did probe a yellow flower were tested until they completed 100 flower choices, starting with the first time they probed a yellow (column 3). Bumblebee colonies vary considerably in size, so the percentage of workers tested from each colony is presented (column 5). In colonies where it was not possible to test 15 workers, we sampled >10% of the total colony worker population.

Colony	Never probed yellow/ n (%)	Probed yellow + 100 choices / n	Total bees tested/ n	workers tested per colony/%
A16		15	15	8.5
A21	2 (12%)	15	17	13.4
A24	9 (43%)	12	21	21.4
A33	3 (17%)	15	18	14.9
A42	1 (6%)	15	16	7.9
A62		15	15	11.2
A65		15	15	13.2
A99		14	14	13.1
A113		15	15	10.8
A126		8	8	11.3
A142		15	15	6.1
A163	2 (12%)	15	17	7.7
A180	1 (6%)	15	16	8.6
A212		15	15	12.3
A228		15	15	11.7
A236	1 (13%)	7	8	10.7
Total	19	221	240	

3.2 Behaviour prior to probing a rewarding flower

At the beginning of training, bees typically chose a high proportion of blue (unrewarding) flowers. On average, bees in 15 of the 16 colonies chose blue flowers at least 9 times in their first 10 flower choices (**Fig 1**). Bees from the other colony (A126) exhibited no clear preference for either flower colour during their first 10 flower choices (colony median = 5; **Fig 1**). This suggests intercolony variation in this trait is discontinuous, and that bees from all colonies (except A126) started their learning in this task from the same high initial level of performance. Irrespective of the initial preference levels, between blue and yellow, the strength of preference persisted in all colonies throughout the period until bees probed their first yellow (rewarding) flower. Thus despite receiving only negative feedback from continuing absence of reward there was no evidence for improvement in task performance during the period prior to the first probing of a yellow flower.

The average number of flower choices made by a bee before probing a yellow flower varied significantly among colonies (Kruskal-Wallis test, $p = 0.009$; **Fig 2**). There were two distinct subgroups of colonies within the overall range of intercolony variation in this trait.

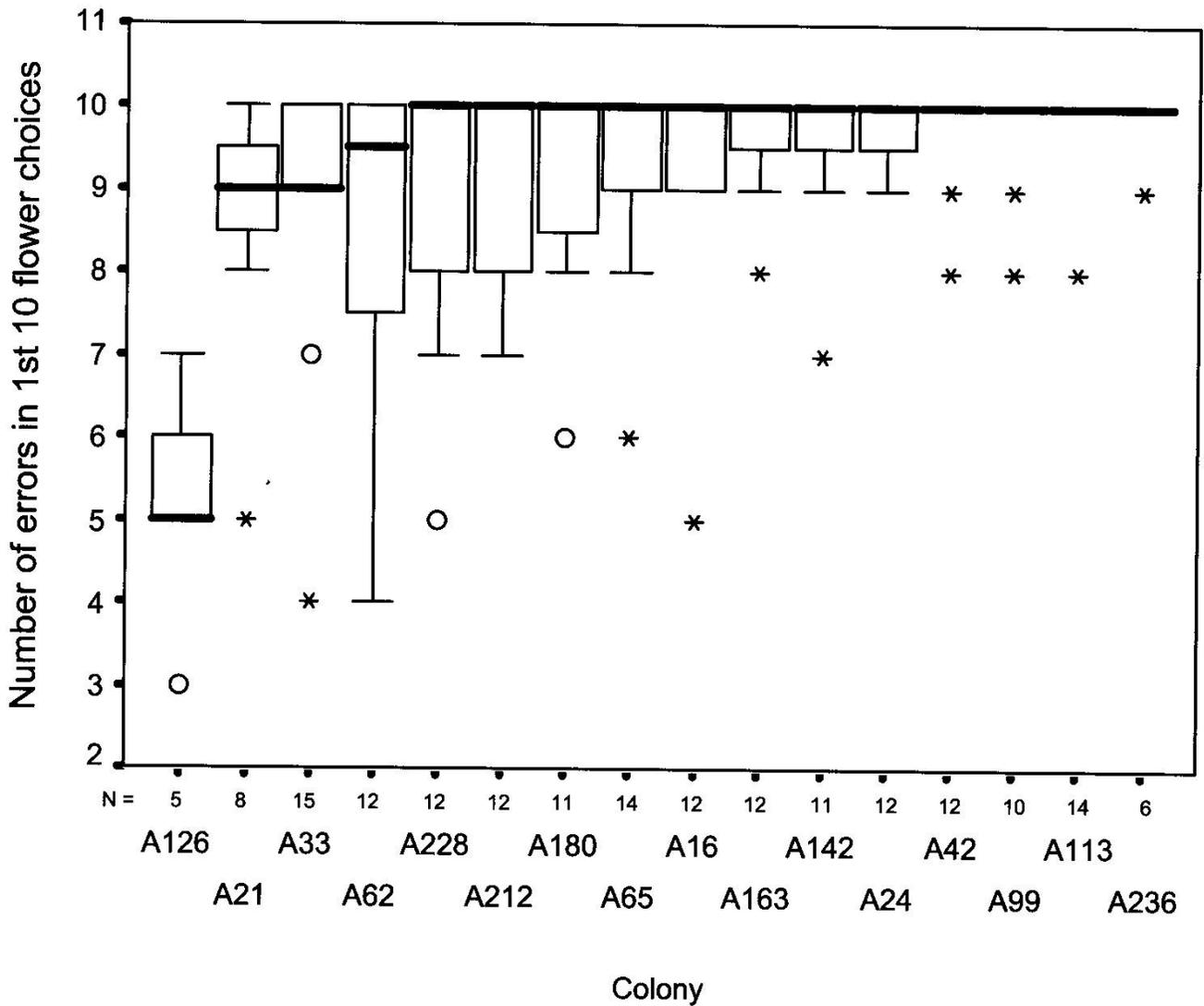


Fig 1: Variation in the number of errors (blue, unrewarding flowers choices) made by bumblebees (*Bombus terrestris audax* Harris 1776 – Hymenoptera: Apidae) during the first 10 flower choices of the learning phase. In each box the thick horizontal bar is the colony median, whilst the lower and upper edges represent the 25% and 75% quartiles respectively. Whiskers indicate the maximum and minimum values that are not outliers. Outliers are represented by open circles, extreme values by asterisks. The number of bees tested in each colony (N) is displayed along the x-axis, and colonies are ranked by increasing colony median value from left to right.

The majority of colonies (A21–A212, n = 14: median range = 11–56), differed significantly from two other colonies (A236 and A24: median range 89–89.5) shown on the right side of Fig 2 (Mann-Whitney U = 885.5, p<0.001). In addition to this appreciable variation in average performance at the colony level, there was also spectacular variation among colonies in the range of flower choices made prior to probing a yellow flower by bees from the same colony. Indeed, the range in number of flower choices made by bees within a single colony varied by more than a factor of ten: from 32 (1–33 choices: A126) to 373 (0–373 choices: A212) choices. It is also noteworthy that although bees in colony A126 showed no strong initial preference for blue over yellow, they did not (on average) probe a yellow flower after fewer flower choices than bees from other colonies (which did show a strong initial preference for blue).

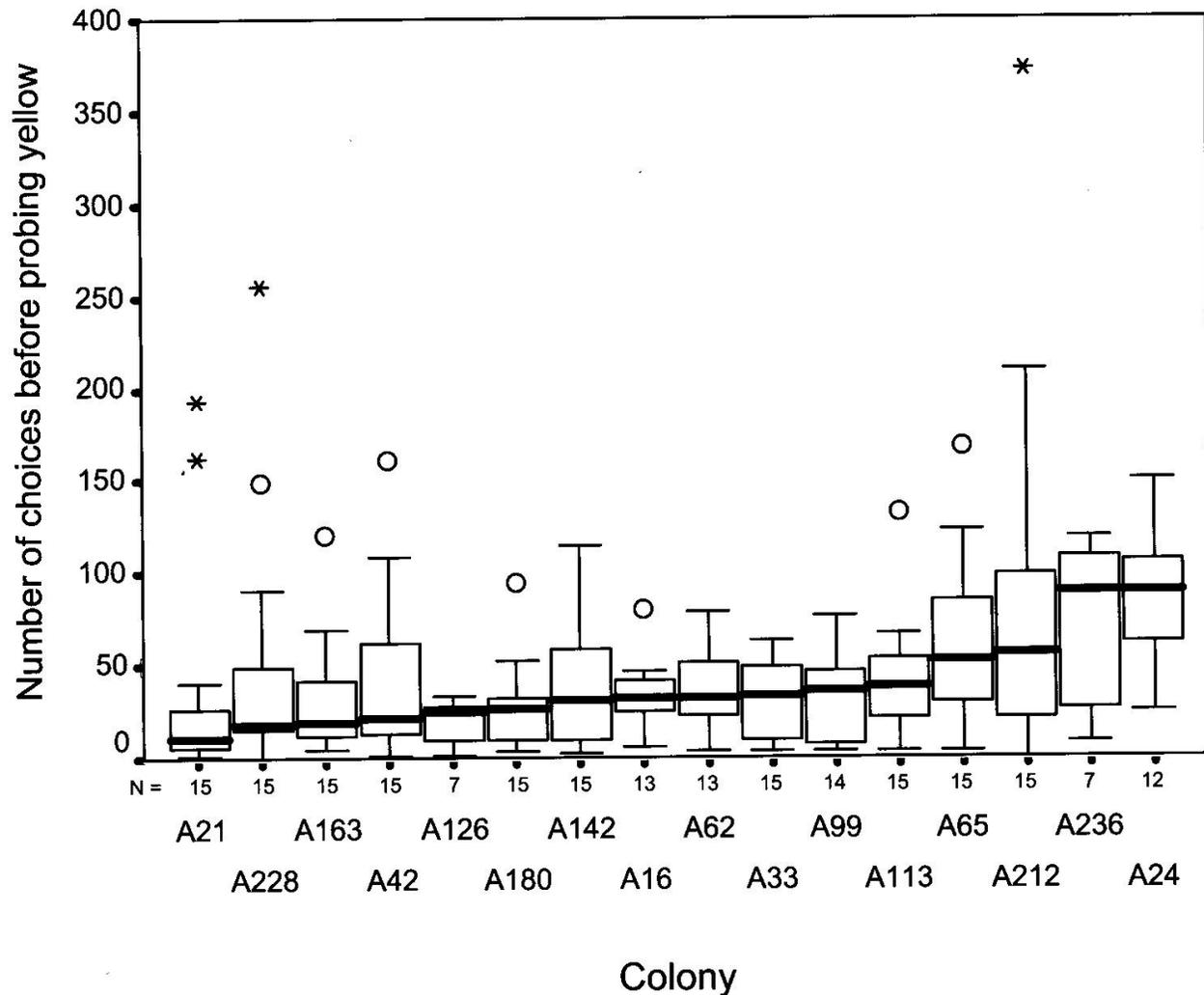


Fig 2: Variation in the number of flower choices made by bumblebees before probing their first yellow (rewarding) flower (*Bombus terrestris audax* Harris 1776 – Hymenoptera: Apidae). In each box the thick horizontal bar is the colony median, whilst the lower and upper edges represent the 25% and 75% quartiles respectively. Whiskers indicate the maximum and minimum values that are not outliers. Outliers are represented by open circles, extreme values by asterisks. The number of bees tested in each colony (N) is displayed along the x-axis, and colonies are ranked by increasing colony median value from left to right.

3.3 Behaviour after probing a rewarding flower

Immediately after probing their first yellow (rewarding) flower, the choice behaviour of bees began to change. All bees became more likely to choose yellow flowers, and this propensity to choose yellow increased in strength as bees sampled more flowers. Indeed, by the end of the experiment, 100 flower choices later, the vast majority of bees showed a very strong preference for yellow over blue: 90% (199 of 221) of bees chose yellow in at least 8 of their last 10 flower choices (including 62% (136 of 221) which chose only yellow), whilst no bees chose less than 5 out of 10 yellow flowers.

Significant variation was observed among colonies in their learning speed for this task (Kruskal-Wallis test, $p = 0.024$), with a very smooth continuous distribution of the average colony learning speed (Fig 3).

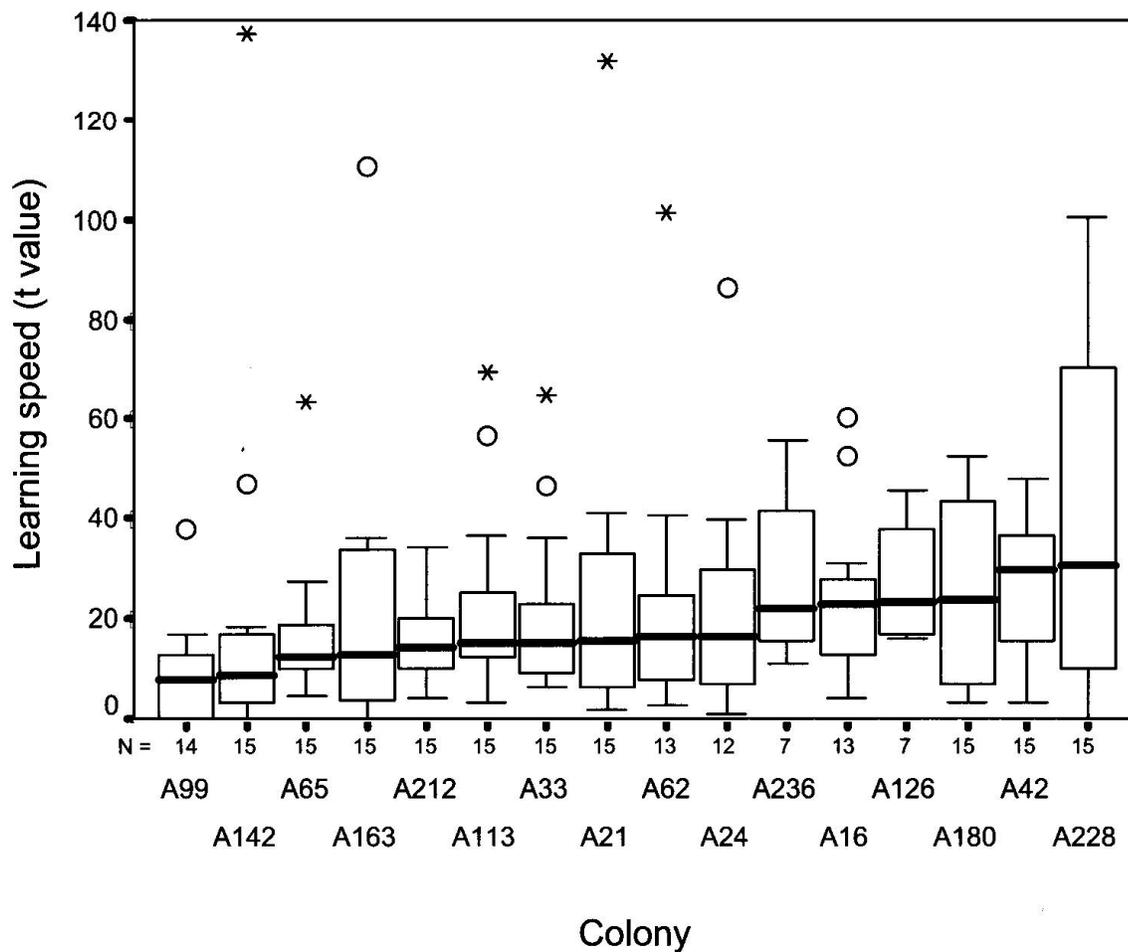


Fig 3: Variation in learning speed (t values) of bumblebees in this task (*Bombus terrestris audax* Harris 1776 – Hymenoptera: Apidae). High values of t correspond to slow learning bees, whereas lower t values indicate faster learners. In each box the thick horizontal bar is the colony median, whilst the lower and upper edges represent the 25% and 75% quartiles respectively. Whiskers indicate the maximum and minimum values that are not outliers. Outliers are represented by open circles, extreme values by asterisks. The number of bees tested in each colony (N) is displayed along the x-axis, and colonies are ranked by increasing colony median value from left to right.

Dunn's multiple comparison post-hoc tests revealed significant differences specifically between colony A99 and A126 ($p < 0.05$), A228 ($p < 0.05$) and A42 ($p < 0.01$) respectively. There were also appreciable intercolony differences in the amount of within-colony variation in learning speeds: bees in some colonies (e.g A65 and A212) showed very uniform learning speed, whilst those in other colonies (e.g A180 and A228) showed appreciable variation. Comparing colony interquartile ranges showed the level of variation in learning speed among bees in colony A228 was almost 7 times greater than that in colony A65.

The level of intercolony variation observed in average learning speeds represents marked differences in the shape of learning curves (**Fig 4**). If amplitude and saturation level are held constant (here $A = 10$, $y_0 = 0$), very large differences in performance between bees with learning speeds equivalent to the average for the fastest (A99), slowest (A228) and intermediate (A21 or A62) colonies were observed.

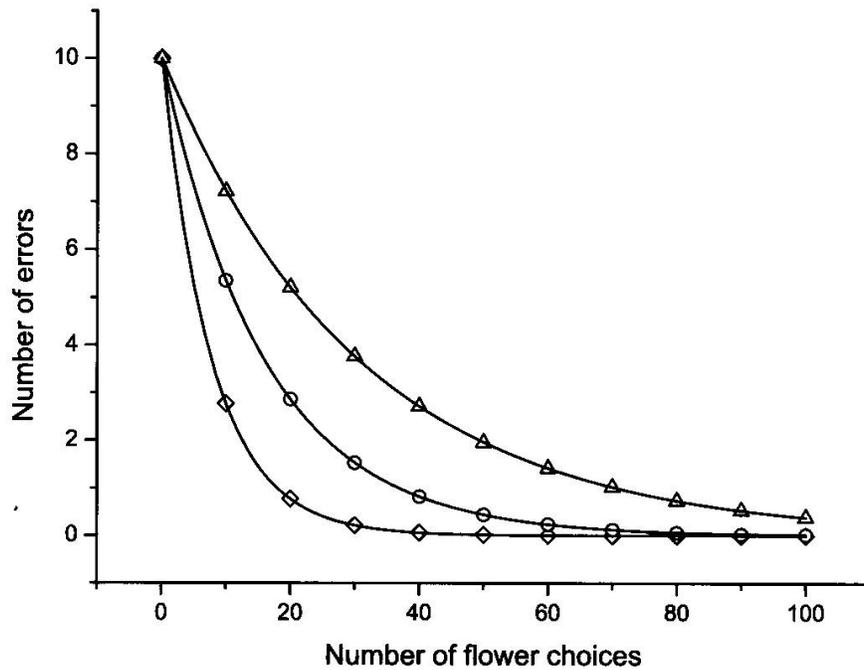


Fig 4: An illustration of how the shape of learning curves relates to learning speed, as expressed by the t value in the equation: $y = y_0 + Ae^{-x/t}$. Curves plotted represent hypothetical learning curves for bees with learning speeds equal to the colony median t value for the fastest (A99, $t = 7.8$: diamonds) and slowest (A228, $t = 30.7$: triangles) learning colonies, and for the population median ($t = 16$, circles). All three curves have amplitude (A) = 10, and saturation performance (y_0) = 0.

For instance, it would take a bee with an average learning speed of the slowest colony ca. 40 flower choices to reach the same performance level reached by a bee with the average learning speed of the fastest colony after only 10 choices, and it would take the first bee far more than 100 flower choices to reach performance saturation, a target the second bee reaches after 50 choices.

The final saturation performance of bees in this learning task (y_0) also showed significant intercolony variation (Kruskal-Wallis test, $p = 0.050$). Whilst the average colony saturation performance for all colonies (except A99) was less than 1 error in 10 flower choices, the majority of colonies contained at least one bee making more than 10% errors (**Fig 5**). There were considerable intercolony differences in the saturation performance levels of bees within the same colony, for example bees in colonies A62 and A99 clearly show much higher levels of variation than bees in colonies A126 or A236. Interestingly, the bees in colony A62 also showed the highest level of intracolony variation in the number of blue flowers chosen in the first 10 flower choices of training (**Fig 1**).

There were no consistent correlations between either bee age (**Tab 2a**) or size (**Tab 2b**) with either learning speed or the number of flower choices made prior to first probing a yellow flower comparing across all colonies tested. However, there were significant correlations between bee age and learning speed in 3 out of 16 colonies (A62, A65 & A236), and a correlation that narrowly missed the 5% significance hurdle ($p = 0.058$) in one other colony (A33: **Tab 2a**). Interestingly, two of these colonies showed a significant negative correlation between age and t value (A62 & A236), whilst the other correlation was positive (A65). There were also significant negative correlations between bee size and t value for colony A65 (**Tab 2b**), and between bee age and number of flower choices made prior to first probing a rewarding flower for colony A163 (**Tab 2a**).

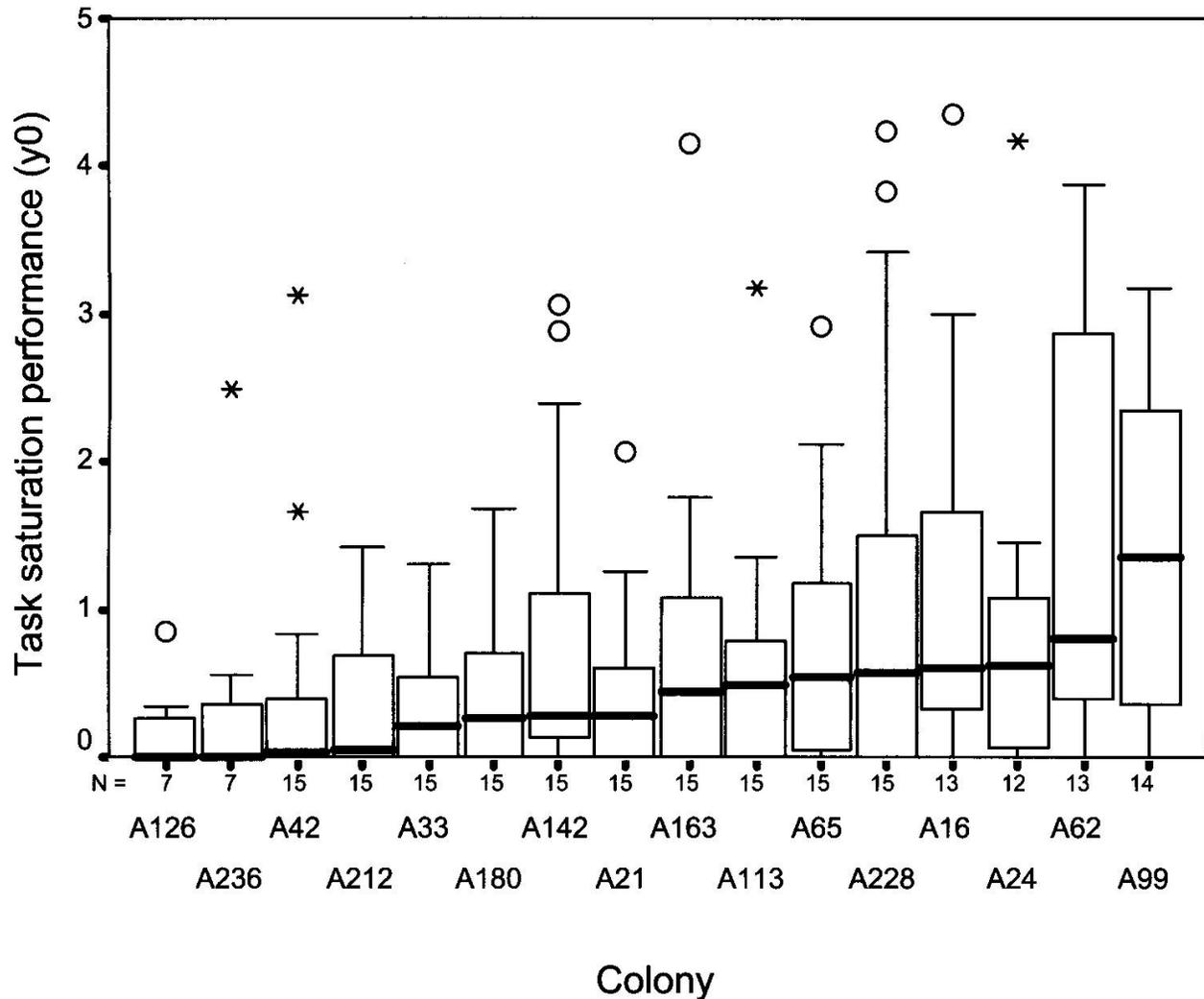


Fig 5: Variation in the number of errors made by bees (*Bombus terrestris audax* Harris 1776 – Hymenoptera: Apidae) at saturation performance (y_0). In each box the thick horizontal bar is the colony median, whilst the lower and upper edges represent the 25% and 75% quartiles respectively. Whiskers indicate the maximum and minimum values that are not outliers. Outliers are represented by open circles, extreme values by asterisks. The number of bees tested in each colony (N) is displayed along the x-axis, and colonies are ranked by increasing colony median value from left to right.

All but one of these significant correlations became non-significant as a result of Bonferroni correction: the negative correlation between bee age and t value remains significant ($r_s = -0.688$, $p = 0.009$) for colony A62 (**Tab 2a**); meaning that, on average, older workers in this colony learned faster (had lower t values).

4 Discussion

The results of our ecologically relevant foraging task show significant intercolony variation in several important aspects of the associative learning process of flower colour as a predictor of floral reward. Perhaps the two most striking of these differences among colonies are the variation in number of flower choices made before bees probed a rewarding flower (the point at which the learning process really appeared to begin), and the variation in learning speed.

Tab 2: Results of Spearman's rank correlation analyses between (a) bee age and (b) bee size on learning speed (t value) and number of flower choices made before probing a rewarding yellow flower for each colony (*Bombus terrestris audax* Harris 1776 – Hymenoptera: Apidae). High t values correspond to slow learning bees, whereas lower t values indicate faster learners. Correlation significance at the 5% level ($\alpha = 0.05$) is indicated by an asterisk, with marginal results indicated with a dot. Significance of correlations after Bonferroni correction ($\alpha = 0.0125$) are indicated by †.

a) bee age	Learning speed/ t value		Number of flower choices before probing yellow		n	
	Colony	r_s	p	r_s		p
A16		0.004	0.991	0.250	0.433	12
A21		0.339	0.217	0.500	0.058 ·	15
A24		0.439	0.153	-0.178	0.580	12
A33		-0.586	0.058 ·	0.030	0.931	11
A42		-0.176	0.529	0.038	0.894	15
A62		-0.688	0.009 *†	-0.234	0.441	13
A65		0.586	0.045 *	0.123	0.703	12
A99		-0.290	0.315	-0.242	0.404	14
A113		-0.142	0.614	-0.135	0.632	15
A126		-0.714	0.071	-0.214	0.645	7
A142		-0.179	0.523	-0.177	0.527	15
A163		-0.041	0.884	-0.538	0.039 *	15
A180		0.309	0.262	-0.409	0.130	15
A212		0.115	0.696	-0.341	0.233	14
A228		-0.271	0.370	-0.083	0.787	13
A236		-0.900	0.037 *	<0.001	1.000	5

b) bee size	Learning speed/ t value		Number of flower choices before probing yellow		n	
	Colony	r_s	p	r_s		p
A16		-0.484	0.094	0.187	0.540	13
A21		0.273	0.324	0.206	0.462	15
A24		-0.118	0.729	-0.200	0.555	11
A33		0.381	0.179	0.363	0.201	14
A42		0.321	0.243	-0.018	0.950	15
A62		-0.313	0.297	-0.358	0.230	13
A65		-0.555	0.032 *	-0.144	0.608	15
A99		-0.499	0.069 ·	0.064	0.828	14
A113		-0.222	0.427	0.248	0.372	15
A126		0.750	0.052 ·	0.071	0.879	7
A142		0.131	0.642	0.504	0.055 ·	15
A163		0.048	0.864	0.154	0.584	15
A180		-0.325	0.237	0.408	0.131	15
A212		0.039	0.889	0.175	0.532	15
A228		-0.061	0.830	-0.121	0.668	15
A236	*	0.536	0.215	0.143	0.760	7

In the early stages of this task, before bees have probed a rewarding flower, the only feedback they receive is negative: either as a result of choosing unrewarding, blue flowers, or failing to probe rewarding, yellow flowers. This feedback appears to have a relatively weak effect on bee flower colour choices by comparison with the strength of their unlearned blue preference. The strong blue preference shown by the British *B terrestris* population in this task is not particularly surprising given that a blue preference has been found in all bumblebee species [CHITTKA & WELLS 2004], including 8 other populations of *B terrestris* [CHITTKA et al 2004, RAINE & CHITTKA 2005a], tested to date. This consistent preference for violet and blue appears to reflect the general profitability of these flower colours in a wide range of habitats [GIURFA, NUNEZ, CHITTKA et al 1995, CHITTKA et al 2004]. Assuming that blue flowers are also generally profitable in the British foraging environment, it also makes adaptive sense that the vast majority of *B terrestris audax* colonies show a very strong unlearned, blue preference.

Why then does a group of colonies, with very similar, high levels of unlearned blue preference, vary so widely in the number of flower choices they make before probing a yellow flower? Perhaps colonies vary in their propensity for other types of exploratory behaviour. It is relatively easy to imagine foraging scenarios which would favour a behaviour that caused bees foraging predominantly on one flower species, to periodically switch species for a few flower visits, allowing it to compare the current reward levels. If the initial species remained the most rewarding, the bee loses very little foraging time exploring the alternative. However, if the second species proved more rewarding, the bee switches to establish a longer sequence of visits [HEINRICH 1979, RAINE & CHITTKA 2005b]. Alternatively, it is possible that strength of colour preference can manifest itself in more than one way. On average, bees from colony A126 made as many flower choices before probing a yellow flower as the majority of other colonies, which functionally suggests that bees in this colony prefer blue (over yellow) flowers. However, there was a big difference in the frequency of blue flowers chosen by bees from colony A126 (median = 5) in the first 10 flower choices in comparison to bees from the other colonies (median ≥ 9).

In general, it seems highly likely that persistently visiting flowers of one species, without receiving any reward, could have serious adverse consequences for the foraging efficiency of bees, and colonies, under natural conditions. Indeed, in this experiment, almost 8% of bees never probed a yellow flower, suggesting that the task was sufficiently challenging to allow to discriminate between bees and colonies. However, it must be considered that the persistence shown by many bees in this experiment to continue choosing blue despite the total absence of reward, could have been influenced by the pre-training. During this time bees were exposed to rewarded flowers which were half blue and half yellow, so had equal opportunity to associate both colours with reward. Instead, what could be happening is that the reward is actually reinforcing the existing, unlearned blue preference, whilst having little or no positive effect of associating yellow with reward. If so, a potential effect of the pre-training could have been to exacerbate within colony trait variation, because there were some differences in the number of pre-training bouts experienced by bees from the same colony. However, no significant effect on the number of flower choices was found, made prior to probing a rewarding flower ($r_s = 0.008$, $p = 0.974$) or learning speed ($r_s = 0.270$, $p = 0.249$) in response to systematic variation in the number of pre-training bouts (5–24 bouts) experienced by 20 bees prior to testing.

Significant variability in the speed with which bumblebee colonies learn colour as a predictor of floral reward was also observed. Since our colonies were raised under carefully controlled, identical environmental conditions, it is likely that these differences are largely genetically determined. This opens up the future possibility to measure the impact of learning performance on fitness in the wild.

The high levels of intercolony variation in learning performance demonstrated here also raise some important methodological considerations of sample size.

Care must be taken when making comparisons between species or populations based on small numbers of colonies. Taking the hypothetical example of sampling the learning performance of two *B terrestris* populations, of which both are as variable as our study population. By sampling a single colony from each population, the likely conclusion would be that populations differed in learning performance. The scale of this difference would depend on where each of our sample colonies fell in their respective population performance distributions. Sample size is also likely to be an issue when examining correlations, such as age or body size effects on behavioural traits. Whilst no overall age effect was found, there were significant correlations between bee age and learning speed in 3 out of 16 colonies (all but one of these became non-significant after Bonferroni correction): two positive and one negative. Thus, randomly selecting a single colony from this population would produce a significant correlation between age and learning speed in 19% of cases. It is intriguing that as many as 3 significant correlations between age and learning speed were found. There is a possibility that this pattern is produced by chance: by testing a sufficiently large number of colonies, a small number might produce an apparent correlation just by stochastic processes. However, it is also possible that the correlations are genuine. Whilst there is no clear age effect on learning speed across all the colonies tested, it is still possible that some colonies show a genetic predisposition for age effects on their learning performance. Indeed, age [RAY & FERNEYHOUGH 1997, LALOI et al 2001] and genotype [BHAGAVAN, BENATAR, COBEY et al 1994] have previously been shown to influence honeybee olfactory learning performance, and LALOI et al [2001] suggested that age-genotype interactions are likely to be important. In visual learning tasks, larger bumblebees (*Bombus impatiens* Cresson 1863) have previously been found to learn more rapidly [WORDEN et al 2005]. In contrast, no overall correlation between body size and learning performance was found in this study, and in the only colony (A65) which showed a significant correlation between size and learning performance, smaller bees learned faster. Overall, while there are obvious practical and economic constraints which tend to keep sample sizes low, it must be considered that even by investing a very significant amount of time and manpower to test 16 colonies, it is unlikely that this study has captured the full range of variation in our trait of interest. When designing experiments it is important to consider the potential significance of variation among, as well as within, colonies when deciding how to allocate finite sampling effort.

Intercolony variation in learning ability has previously been demonstrated in honeybees [CHANDRA, HOSLER & SMITH 2000, LATSHAW & SMITH 2005]. However, these studies compared variation in the performance of selectively bred lines, in a species which has been domesticated for several thousand years [CRANE 2004]. The large scale study reported here represents the first to quantify the intraspecific variation in learning performance existing within a natural bee population. As the colony is the unit of selection in social insects, the significant levels of intercolony variation demonstrated here represent the raw material upon which selection for learning ability might act. This forms the basis from which to explore the potential adaptive value and constraints imposed on such variation in the economy of nature.

5 References

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 BARTH F G & SCHMID A (Eds.): **Ecology of Sensing**. – [341 Seit, 100 SW-Bilder, 160 x 240 mm, Hartkarton Ebd.]. – **Publ:** Springer-Verlag, Berlin Heidelberg New York 2001; **ISBN:** 3-540-66901-9; **Pr:** US \$ 142,-. [EGR-Nr 2.823]

Adaptives Verhalten der Tiere basiert auf relevanter Information. Information wird dem Zentralnervensystem durch Sinnesorgane vermittelt. Dabei sind Sinnesorgane keine passiven Fenster in die Umwelt, sondern sie spielen eine sehr aktive Rolle bei der Filterung und Kategorisierung der physikalischen Umgebung der Lebewesen. Dabei werden die Eigenschaften der Sinnessysteme im Laufe der Evolution den physikalischen Eigenschaften der Reizfelder und der biologischen Relevanz für die Lebewesen angepasst. Diese Sicht wurde bereits 1909 von JAKOB VON UEXKÜLL propagiert, aber es dauerte nahezu ein Jahrhundert, bis diese Gedanken als „Sensorische Ökologie“ eine erfolgreiche Renaissance erlebten.

Bisher wurden wenige Bücher zur Sensorischen Ökologie geschrieben, was angesichts der rasch ansteigenden Menge an Detailwissen zu bedauern ist.

Das Buch ‚*Ecology of Sensing*‘, das von den beiden Wiener Zoologen FRIEDRICH G BARTH und AXEL SCHMID herausgegeben wurde, ist ein höchst überfälliger und sehr gelungener Beitrag zu diesem wichtigen Teilgebiet der Tierphysiologie, hervorgangen aus einem Symposium zum Thema, das 1999 in Wien stattgefunden hat.

In eigenen Kapiteln haben 25 der führenden Wissenschaftler niedergelegt, was es zum Kenntnisstand zu sagen gibt. Die Herausgeber haben diesen Kapiteln eine erklärende Einleitung vorangestellt und alle Beiträge zusammengefaßt in den Abteilungen ‚*General Aspects*‘, ‚*Sound and Hearing*‘, ‚*Medium Flow and Vibrations*‘, ‚*Light and Vision*‘, ‚*Odors and Chemoreception*‘, ‚*Hygro- and Thermoreception*‘ and ‚*Magnetic Field and Electoreception*‘. Damit sind alle Gebiete vertreten, auf denen die am weitest reichenden Durchbrüche in ‚*Sensorischer Ökologie*‘ zu verzeichnen sind. Jedes Kapitel ist mit einer ausführlichen Referenzenliste ausgestattet; für das gesamte Buch ist ein Schlagwort-Index angehängt. Das Buch eignet sich nicht nur für Spezialisten, sondern es kann auch für breit interessierte Biologen, nicht zuletzt als Basis für entsprechende Lehrveranstaltungen empfohlen werden.

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