RESPONSE

Bumblebee colour patterns and predation risk: a reply to Owen (2014)

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It has been observed that sympatric bumblebees species assemblages often exhibit similarities in their colour patterns (Plowright & Owen, 1980; Williams, 2007). An attractive suggestion is that this inter-specific similarity in coloration could result from learnt avoidance by avian predators: if a bird learns from adverse experience that a certain colour pattern is associated with a painful sting, it might subsequently generalize this experience to avoiding similar patterns displayed by other bee species, potentially leading aposematic insects to form local mimicry rings (Plowright & Owen, 1980; Williams, 2007). We recently tested this idea with reciprocal transplant experiments using bumblebees in which we compared the worker loss rates of foreign and local colour pattern variants. If local mimicry rings protect the local colour variant, we would expect foreign individuals, with unusual colour patterns, to be exposed to higher predation than local bee populations whose colour patterns should be familiar to local predators (Stelzer et al., 2010). The hypothesis that foragers that differ in coloration from the local native population would suffer higher predation risk was not upheld by our experiments. However, Owen (2014) now disputes the conclusions we draw from our empirical study. Having undertaken a re-analysis of our published data, he proposes a number of additional, alternative hypotheses, as yet unsupported by empirical evidence, to explain the perceived discrepancy between our data and the mimicry ring hypothesis. Owen suggests that if *all* the additional conditions he outlines apply, our data might be consistent with the mimicry ring hypothesis. Here, we carefully explore Owen's hypotheses and provide evidence to reject them. Other explanations are therefore needed to explain the apparent convergence of bumblebee colour patterns in certain locations and its impact on predation rates.

Insectivorous birds do not initially avoid aposematic insects but swiftly learn to associate visual patterns with aversive reinforcement (Mostler, 1935; Skelhorn & Rowe, 2006; Chittka & Osorio, 2007). Such learnt avoidance has been suggested to explain the observation that bumblebees from different species sometimes appear to display similar colour patterns, even though there is a remarkable diversity in coat colour both within and between bumblebee species (Williams, 2007). Support for this hypothesis requires more than just quantification of bumblebee colour patterns in various locations and an assessment of their apparent similarity from an avian predator's perspective; it also requires an empirical demonstration that bumblebees with different colour patterns experience differential predation, depending on how similar their appearance is to the predominant local aposematic colour pattern(s). We explored whether diversity in bumblebee colour patterns, found in several locations in which different bee colorations naturally predominate, affects the loss rate of workers during foraging – some of which must be because of predation (Stelzer et al., 2010). However, although we found that there were pronounced differences in loss rates between bumblebees with different colour patterns in all locations tested, the native, local colour patterns did not provide consistently higher safety from being lost during foraging than non-native coloration (Stelzer et al., 2010).

Owen (2014) observes that during summer (after fledging), the number of young, inexperienced birds can outnumber older, experienced individuals (King, Farner & Mewaldt, 1965). During this period, one might not expect local aposematic insects to be better protected because naïve birds will initially sample all colour patterns equally. Our data allow us to test this hypothesis as two of the experiments were conducted during summer months (July and August in the UK and Germany) and compared loss rates of bees presenting native-like colour patterns with foreign ones (Stelzer et al., 2010). In the UK summer, the loss rate for the native-like colour pattern (*Bombus terrestris dalmatinus*) was consistently lower than for the non-native pattern (*Bombus terrestris canariensis*) in both years, though this difference was not statistically significant in our analysis (Stelzer et al., 2010). In Owen's re-analysis of the same data using a different statistical test, he found a significant advantage for the native-like colour pattern (Owen, 2014) – refuting his own suggestion that there

should be no difference associated with bumblebee colour pattern in summer. In the German summer, we showed a statistically significant effect of bumblebee population membership (associated with colour coat), with the local native bees (*Bombus terrestris terrestris*) showing higher loss rates than those with foreign colour patterns (both *Bombus terrestris sassaricus* and *B. t. canariensis*). However, using a different quantitative evaluation, Owen finds that the difference in loss rates among populations misses the 5% significance hurdle. Although the actual *P* values for these *t*-test comparisons are not reported (Owen, 2014), he acknowledges the difficulty with a potential type II statistical error, given the complications involved with using *t*-tests for low sample sizes. Nonetheless, he concludes that the lack of a significant effect (in Germany) supports his hypothesis that in summer insectivorous birds, on average, do not discriminate between colour variants.

Inexperienced birds learn to avoid bumblebees after very few learning trials and form long-lasting memories of the adverse experiences associated with attempting to consume stinging insects (Mostler, 1935; Skelhorn & Rowe, 2006). In addition, although juvenile bird mortality is often high, most of the mortality occurs immediately after fledging (Ringsby, Saether & Solberg, 1998). Therefore, by autumn, most insectivorous birds should have had the opportunity to sample a variety of insects and have learnt to avoid stinging ones. Curiously, Owen (2014) makes the same predictions for data collected in November to December in Sardinia (autumn) with those from the summer in Germany (indeed 'summer' and 'autumn' appear to be used interchangeably in his article). Again, using a modified quantitative analysis, Owen finds no difference in loss rates associated with colour pattern for data collected in Sardinia (even though we had demonstrated such a difference using appropriate statistics: Stelzer *et al*., 2010) and concludes that this is in line with the prediction that naïve birds are numerically dominant and do not discriminate between bumblebee colour forms. Surprisingly, Owen makes no mention that the mean loss rates differ by at least a factor of 3 among populations with different colour patterns tested in Sardinia (Stelzer *et al*., 2010).

Using the results of his re-analysis, Owen proceeds to evaluate the number of cases in which our data support his hypothesis. In Owen's (2014) Table 3, his prediction is not met in three out of eight tests. Turning to Owen's (2014) Table 4, his predictions are not met in seven out of 12 tests. Given the somewhat equivocal support for his predictions, we are surprised Owen feels justified in concluding our interpretations of these data are incorrect. After finding that there are so many cases in which the data do not fit his hypothesis, Owen proposes a number of alternative explanations for the remaining discrepancies. He suggests that one reason that the Sardinian population might cope poorly in Germany is because of the colder and wetter weather. This seems an unlikely explanation as the experiments in Southern Germany were conducted at the height of summer, a period with the highest temperatures and minimal rainfall in the excellent wine-growing region around Würzburg. Owen then suggests eliminating the Sardinian bumblebees (*B. t. sassaricus*) altogether from comparisons. Eliminating one of three populations from a reciprocal transplant experiment seems a peculiar suggestion, but his rationale is that 'there is a problem with the Sardinian bees' (Owen, 2014). Again, this suggestion seems unlikely as *B. t. sassaricus* colonies produce larger workers, and those workers collect nectar more efficiently than *B. t. terrestris* in six out of nine comparisons of foraging performance in the same environment (even when body size is removed as a factor: Chittka, Ings & Raine, 2004; Ings, Schikora & Chittka, 2005). There is no published evidence that this population is particularly unfit, making it hard to justify excluding these bees from comparisons.

Even if we accept Owen's suggestion to exclude the Sardinian population from all comparisons, there remains the complication that non-native *B. t. terrestris* perform better than non-native *B. t. canariensis* in Sardinia in autumn (November to December). To explain this anomaly, Owen suggests that migratory birds residing in Sardinia at this time of year might have remembered their previous encounters with *B. t. terrestris* on the continent. However, this would seem to contradict Owen's own argument that there should be no difference associated with colour pattern at this time of the year as naïve, inexperienced birds should dominate predation pressure on insects.

Explaining away outliers, or removing entire datasets, that depart from one's favoured hypothesis is potentially unsound scientific practice. In our original study, we adopted a strictly data-driven approach: we used a large dataset collected to investigate differences in foraging performance among *Bombus terrestris* populations in common environments (Ings *et al*., 2005). The data were not collected to explore any hypothesis about mimicry, which removes any possibility for experimenter bias during their collection. Adjusting a hypothesis after finding discrepancies between the original idea and the pattern shown by the data is fine; therefore, Owen's suggestion that there might be no adaptive benefit for any particular aposematic coloration at certain times of year as fledgling birds will sample any type of insects is worth exploring. However, we conclude that support for Owen's modification to the local mimicry ring hypothesis from our data is not strong (see previous discussion).

It is likely that there are many factors that introduce noise alongside the pressures exerted by insectivores on insect aposematic coloration (so that there are many conditions in which colour patterns have little influence or that they matter in ways unrelated to predation). However, if the idea that protection is afforded by joining an aposematic mimicry ring is correct, on average, one should still see a native, local colour pattern providing a protective advantage in most cases. It is possible that we might see an effect if we collected data over many seasons, additional locations and multiple times of the year, etc. At the time of writing, our dataset is the largest and most complete investigation on this question in the literature, and it does not contain unambiguous pointers to support the mimicry ring hypothesis. Scientists' efforts should now focus on collecting additional data rather than constructing further *ad hoc* arguments to explain away discrepancies between empirical data and existing theory.

Acknowledgments

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