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Evolution of kin recognition mechanisms in a fish

Timothy J. A. Hain[1] · Shawn R. Garner[1] · Indar W. Ramnarine[2] ·
Bryan D. Neff[1]

Abstract Both selection and phylogenetic history can influence the evolution of phenotypic traits. Here we used recently characterized variation in kin recognition mechanisms among six guppy populations to explore the phylogenetic history of this trait. Guppies can use two different kin recognition mechanisms: either phenotype matching, in which individuals are identified based on comparison with a recognition template, or familiarity, in which individuals are remembered based on previous interactions. Across the six populations, we identified four transitions in recognition mechanism: phenotype matching evolved once and was subsequently lost in a single population, whereas familiarity evolved twice. Based on a molecular clock, these transitions occurred among populations that had diverged on a timescale of hundreds of thousands of years, which is two orders of magnitude faster than previously documented transitions in recognition mechanisms. A randomization test provided no evidence that recognition mechanisms were constrained by phylogeny, suggesting that recognition mechanisms have the capacity to evolve rapidly, although the specific selection pressures that may be contributing to variation in recognition mechanisms across populations remain unknown.

✉ Bryan D. Neff
bneff@uwo.ca

1 Department of Biology, University of Western Ontario, London, ON N6A 5B7, Canada

2 Department of Life Sciences, The University of the West Indies, St. Augustine, Trinidad and Tobago

Introduction

The traits we observe in modern species can be influenced by both selection and phylogenetic history (e.g. Wilson 1975). Selection can favour adaptations that better match individuals to their environments, but evolutionary constraints can limit adaptation if populations lack genetic variation on which selection can act. As a consequence of evolutionary constraints, many traits show evidence of phylogenetic signal, in which closely related taxa are more similar than distantly related taxa (Blomberg et al. 2003). Interestingly, the strength of phylogenetic signal has been shown to be lower for behavioural traits than for other traits (Blomberg et al. 2003), suggesting that behaviour may be evolutionarily labile and thus may be a key component to adaptation in novel or rapidly changing environments (Hardy 1965; Lister 2014).

Kin recognition allows individuals to respond adaptively to the presence of genetic relatives, with familiarity and phenotype matching being the most common mechanisms (Mateo 2004). Familiarity is based on prior association among family members, and when it is used as a recognition mechanism, individuals remember conspecifics encountered early in life, particularly in the vicinity of the natal area (e.g. the nest), and later treat these individuals as related. With phenotype matching, individuals instead use aspects of the phenotype such as odour, sound, or appearance of conspecifics encountered early in life to build a "kin template". Later, putative kin are compared to the kin template and treated as related or unrelated based on the degree of similarity (Holmes and Sherman 1982;

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Mateo 2004). Although kin recognition mechanisms have been widely studied, the questions of how these mechanisms evolved remain largely unresolved.

Guppies (*Poecilia reticulata*) have emerged as a model system for the study of evolution because populations have repeatedly experienced convergent evolution in behavioural, morphological, and life history traits in response to differences in local predation pressure (Seghers 1974; Endler 1983; Reznick et al. 1997) and other ecological factors (e.g. Grether et al. 2001). Hain et al. (2016) recently characterized variation in kin recognition mechanisms across six guppy populations in Trinidad. The authors found that one population used both familiarity and phenotype matching, one used only familiarity, three used only phenotype matching, and one population did not use either mechanism. Here we take advantage of this variation in recognition mechanism to provide the first test of the relationship between kin recognition mechanisms and phylogeny. We use mitochondrial control region sequences to assess the phylogenetic relationships among the guppy populations and fit the observed recognition mechanisms to the resulting tree. We then use a randomization routine to test whether or not phylogenetic signal can explain the pattern of variation in kin recognition mechanisms across populations.

Materials and methods

The guppy populations we studied are found in Trinidad's three major river drainage systems: the west-flowing Caroni drainage (Tunapuna: 10°42′N, 61°21′W; Upper Aripo: 10°42′N, 61°12′W; Lower Guanapo: 10°39′N, 61°12′W; Supplementary material, Figure S1); the northern drainages (Upper Yarra: 10°47′N, 61°21′W; Paria: 10°45′N, 61°16′W); and the east-flowing Oropouche drainage (Lower Oropouche: 10°40′N, 61°08′W). For each of the six populations, we obtained mitochondrial control region sequences using a combination of previously published sequences and field-collected samples (see supplementary materials for additional sampling details). In total, control region sequences were obtained from 41 guppies, including 5–13 fish from each population.

We previously characterized the recognition mechanisms used by guppies from these six populations (Hain et al. 2016). Briefly, the recognition mechanism of juvenile guppies was tested with a series of dichotomous choice trials to evaluate the association preference of a focal fish based on the effects of familiarity or relatedness. That is, in trials that tested the effects of familiarity, one stimulus fish was familiar to the focal fish, the other stimulus fish was unfamiliar, and both fish shared the same relatedness to the focal fish (either related or unrelated). In trials that tested

the effects of relatedness, one stimulus fish was related to the focal fish, the other stimulus fish was unrelated, and both fish shared the same level of familiarity to the focal fish (either familiar or unfamiliar). Guppies from a particular population were characterized as using familiarity if fish from that population had a significant association preference for familiar over unfamiliar individuals, and characterized as using phenotype matching if fish from that population had a significant association preference for related over unrelated individuals. We found that guppies from the Paria used both familiarity and phenotype matching, guppies from the Upper Aripo used only familiarity, guppies from the Lower Guanapo, Tunapuna, and Upper Yarra used only phenotype matching, and guppies from the Lower Oropouche did not use either kin recognition mechanism.

The phylogenetic relationships among populations were determined by analysing the control region sequences using MrBayes version 3.2.2 (Ronquist et al. 2012). This analysis used the program's recommended settings and included a sequence from the closely related *Poecilia picta* to root the tree (GenBank accession: AF033053). The resulting tree indicated that all sequences from the same population grouped together (Supplementary material, Figure S2), so the relationships among populations were collapsed into a single consensus tree.

We first mapped the observed kin recognition mechanisms for the six guppy populations onto the consensus tree to determine the minimum number of evolutionary transitions between recognition mechanisms. We then used a randomization routine to determine the expected number of evolutionary transitions in the absence of phylogenetic signal. This routine incorporated the variance in the kin recognition observations (i.e. individual kin recognition trials) by resampling observations within each population with replacement, with one-tailed t tests used to determine whether there was evidence for each kin recognition mechanism (as in Hain et al. 2016). Phylogenetic signal was removed by randomizing the location of populations on the consensus tree, after which we calculated the number of evolutionary transitions needed to explain the simulated data. The randomization routine was repeated 10,000 times to produce a distribution of the expected number of evolutionary transitions in the absence of phylogenetic signal. The probability of obtaining the observed number of transitions in the absence of phylogenetic signal was then calculated as the proportion of the simulated data that were less than or equal to the observed value.

To estimate the timescale over which recognition mechanisms have changed among populations, we used a molecular clock based on the mitochondrial control region sequences. This analysis was performed in BEAST 1.7 (Drummond et al. 2012). We set the mutation rate

parameter using a lognormal distribution based on previous estimates of the control region's mutation rate from nine fish species (Burridge et al. 2008). The time to the most recent common ancestor was then estimated for each node in the population tree based on simulations with 10 million generations in which values were logged every 1000 generations. Median and 95% confidence intervals for the node ages are presented based on the distribution of the 10,000 logged values.

Results

Our phylogenetic analysis showed that the guppies from the Oropouche drainage were the most genetically distinct, having diverged from the other populations 330,000–2,970,000 years ago (95% CI, Fig. 1). Guppies from the northern drainage diverged next, with the Paria population diverging 110,000–1,010,000 years ago, followed by the Upper Yarra population 80,000–760,000 years ago. Lastly, the three populations in the Caroni drainage (Lower Guanapo, Tunapuna, Upper Aripo) diverged from each other 30,000–350,000 years ago. Mapping the observed recognition mechanisms onto the consensus phylogenetic tree revealed that the most parsimonious explanation was that phenotype matching evolved once and was subsequently lost in one population, whereas familiarity evolved twice. The most rapid
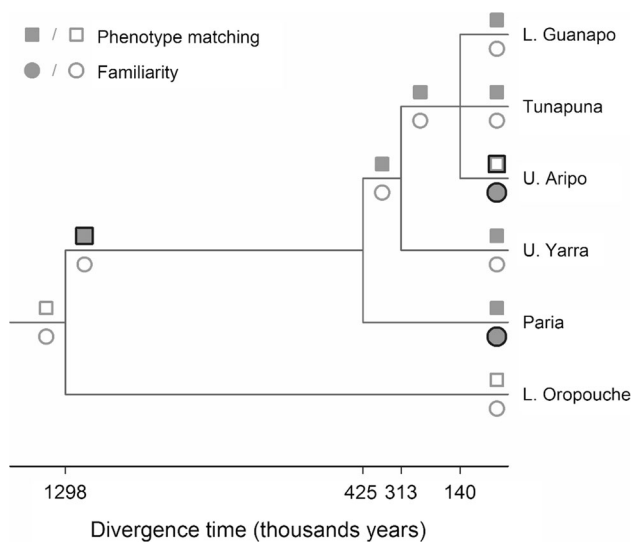
Fig. 1 Phylogenetic tree based on the mitochondrial control region, as well as the kin recognition mechanism for each branch and node in six populations of guppies (*Poecilia reticulata*). The branch lengths of the tree are scaled based on a molecular clock, with the median age for each node indicated on the *axis*. Phenotype matching is represented with *squares* and familiarity with *circles* (present = *filled*, absent = *open*). Transitions between states are highlighted with a *black outline*
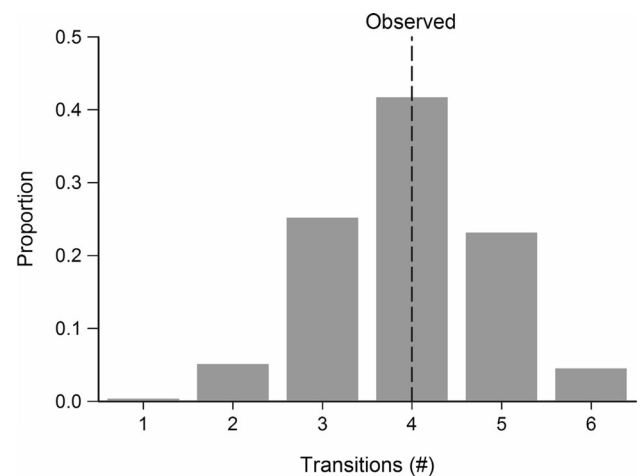
Fig. 2 Histogram with the results of a simulation analysis that determined the expected number of transitions between kin recognition mechanisms for six populations of guppies (*Poecilia reticulata*), assuming that recognition mechanisms were independent of phylogeny. The observed number of transitions was not significantly different from the expected number in the absence of phylogenetic signal ($P = 0.72$)

transitions in recognition mechanisms occurred in the Upper Aripo population, which both gained familiarity and lost phenotype matching within at most 350,000 years of diverging from the other Caroni drainage populations (Fig. 1). Overall, the four total transitions in recognition mechanisms that we observed did not differ from the number expected in the absence of a phylogenetic signal ($P = 0.72$; Fig. 2).

Discussion

There is considerable interest in the evolution of kin recognition systems. Previously, the finest scale that variation in recognition mechanisms had been observed was across species within a genus (Lizé et al. 2014), with associated divergence times conservatively estimated on the order of tens of millions of years (Gao et al. 2007). We now show that kin recognition mechanisms have repeatedly evolved across guppy populations, with associated divergence times on the order of hundreds of thousands of years. Thus, we have provided evidence for the fastest evolutionary divergence in recognition mechanisms to date. Interestingly, we found that recognition by phenotype matching had a single origin, whereas familiarity emerged in two separate lineages. Although based on a small number of populations, it is plausible that kin recognition by familiarity can evolve more rapidly than recognition by phenotype matching, as familiarity may be pre-adapted as an extension of existing social behaviours that allow individuals to identify

and remember conspecifics (Mateo 2004). Phenotype matching on the other hand requires the evolution of both a recognition template and the subsequent development of recognition behaviours (Holmes and Sherman 1982). Furthermore, we found that the evolution of phenotype matching does not require the pre-existence of familiarity as a recognition mechanism, which suggests that the two mechanisms involve independent cognitive processes. Thus, it is unlikely that the phenotype matching mechanism has evolved from a modification to a pre-existing familiarity recognition mechanism. The relationship between the development of familiarity and phenotype matching, as well as the relative rates of evolution of these two mechanisms, deserves further investigation.

Behavioural traits have been shown to be more evolutionary labile than other biological traits (Blomberg et al. 2003), and there are numerous examples of rapid evolution of behavioural traits in response to changing selection pressures (e.g. Magurran et al. 1992; Singer et al. 1993). Therefore, it is not really surprising that the patterns of recognition mechanisms that we observed across populations did not show evidence of phylogenetic signal, suggesting that recognition mechanisms are also evolutionarily labile. However, the selection pressures that might be shaping variation in recognition mechanisms are not yet well resolved. Recent studies comparing closely related taxa suggest that diet and social behaviour (Lizé et al. 2014) or mating system (Hain et al. 2016) are important in determining recognition mechanisms. Ultimately, additional studies that examine recognition mechanisms within the context of phylogeny and ecology are needed to more fully understand the evolution of this important behavioural trait.

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