

EVOLUTION OF GENETIC INTEGRATION BETWEEN DISPERSAL AND COLONIZATION ABILITY IN A BIRD

Renée A. Duckworth^{1,2,3} and Loeske E. B. Kruuk³

¹*Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138*

²*E-mail: rad3@email.arizona.edu*

³*Institute for Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JT United Kingdom*

Received August 21, 2008

Accepted November 14, 2008

Discrete behavioral strategies comprise a suite of traits closely integrated in their expression with consistent natural selection for such coexpression leading to developmental and genetic integration of their components. However, behavioral traits are often also selected to respond rapidly to changing environments, which should both favor their context-dependent expression and inhibit evolution of genetic integration with other, less flexible traits. Here we use a multigeneration pedigree and long-term data on lifetime fitness to test whether behaviors comprising distinct dispersal strategies of western bluebirds—a species in which the propensity to disperse is functionally integrated with aggressive behavior—are genetically correlated. We further investigated whether selection favors flexibility in the expression of aggression in relation to current social context. We found a significant genetic correlation between aggression and dispersal that is concordant with consistent selection for coexpression of these behaviors. To a limited extent, individuals modified their aggression to match their mate; however, we found no fitness consequences on such adjustments. These results introduce a novel way of viewing behavioral strategies, where flexibility of behavior, while often aiding an organism's fit in its current environment, may be limited and thereby enable integration with less flexible traits.

KEY WORDS: Dispersal polymorphism, genetic covariance, heritability, temperament.

Interactions among traits often increase organismal fitness or performance leading to selection for functional integration and trait coexpression (Lande 1979, 1980; Cheverud 1996). In quantitative genetic terms, selection for functional integration among traits at the level of the individual can produce genetic correlations between traits at the level of the population (Houle 1991; Atchley et al. 1994; Cheverud 1996). Once formed, genetic correlations can constrain the independent evolution of traits and, in the short term, can significantly affect the response to selection (Roff 1997; Lynch and Walsh 1998; Riska 1989). Thus, identifying patterns of phenotypic and genetic covariance among traits can provide important insight into the processes underlying the evolution of complex phenotypes—a topic that remains a funda-

mental problem in evolutionary biology (Schlichting and Pigliucci 1998; Wagner 2001). In particular, investigating the evolution of integration among traits that have heterochronic patterns of variance expression during their ontogeny can provide key insights into the mechanisms underlying the coexpression of traits (Atchley 1984, 1986; Riska 1989; Cheverud 1996). For example, expression of some traits remains constant since early in ontogeny, whereas other traits retain flexible and context-dependent expression throughout an individual's life (West-Eberhard 2003; Sgrò and Hoffman 2004; Duckworth 2009). Yet, even traits that strongly differ in expression of developmental variability are frequently closely integrated in their function and consistent selection for such phenotypic coexpression should favor the

evolution of their developmental and, ultimately, genetic integration (Lande 1979, 1980; Cheverud 1982, 1996; Atchley and Hall 1991; Wagner 1996).

Behavioral traits, with their ubiquitous context-dependent adjustment, are often highly flexible in their expression and yet, are frequently functionally integrated with less flexible aspects of the phenotype, such as morphology. For example, in alternative mating tactics, a suite of sexual and parental behaviors are often linked to distinct morphologies (Emlen 1997; Badyaev and Hill 2002; Tuttle 2002). The common occurrence of such adaptive behavioral tactics—in which multiple behavioral, morphological, and physiological traits are closely coordinated in their expression (Shuster and Wade 2003)—emphasizes that the evolution of functional integration between highly flexible and invariant traits is often resolved. One possible mechanism for the resolution of this paradox is that integration may occur early in ontogeny when the general mean level of expression of both traits is determined, but one or both traits retains some flexibility in expression around this mean during adulthood. Such coordinated expression could be due to shared dependence of traits on either genetic or environmental factors during ontogeny (e.g., Badyaev 2002, 2004). Regardless of the source, consistent selection for trait coexpression that accompanies the evolution of adaptive behavioral strategies should result in integration of ontogenies of traits and, ultimately, the evolution of genetic integration between individual traits.

Distinct dispersal strategies are examples of behavioral strategies in which a behavior that is expressed early in life—the juvenile dispersal decision—is closely integrated with a suite of morphological, physiological, behavioral, and life-history traits that enhance colonization ability and often retain flexibility in expression throughout an organism's life (e.g., Roff and Fairbairn 2001). Such strategies are commonly described in insects that express winged dispersive and wingless nondispersive morphs (Harrison 1980; Roff 1986; Zera and Denno 1997). In other animals, particularly in vertebrates, the propensity to disperse is often linked to a suite of behavioral traits rather than to distinct morphologies (Gaines and McClenaghnam 1980; Toonen and Pawlik 2001; Skjelseth et al. 2007). For example, in birds, highly dispersive individuals are often more exploratory and aggressive—behaviors that can enhance survival and competitive ability in a novel environment (Dingemanse et al. 2003; Duckworth and Badyaev 2007). Although a link between dispersal and colonization behavior might be functionally analogous to distinct dispersal morphs of insects (Toonen and Pawlik 2001), it is not known whether the often documented close integration of behavioral phenotypes and dispersal is underlain by a genetic correlation among its components similar to morphological components of dispersal strategies (Fairbairn and Roff 1990).

Studying genetic integration of dispersal and colonization behaviors requires an empirical system in which behavioral traits

can be studied across multiple generations and in which the lifetime fitness consequences of phenotypic coexpression of dispersal and colonization behavior are known. Western bluebirds (*Sialia mexicana*) have two distinct behavioral strategies that integrate aggression and natal dispersal (hereafter, dispersal)—nonaggressive males tend to remain in their natal population whereas aggressive males tend to disperse and colonize new areas (Duckworth and Badyaev 2007; Duckworth 2008). Expression of aggression, although repeatable within an individual, retains some flexibility throughout adulthood (Supporting Fig. S1), whereas dispersal is a one-time decision made during the juvenile period (Guinan et al. 2000). Close integration of dispersal strategy and aggression is under strong natural selection because aggressive males outcompete less-aggressive males for nesting sites and territories making them better at colonizing new populations, whereas nonaggressive males have greater success in acquiring a territory through cooperation with relatives in their natal population and ultimately have higher fecundity because of their greater investment in parental care (Duckworth 2006a,b; 2008). Moreover, the strong ecological importance of integration of these behaviors is emphasized by their role in facilitating the expansion of western bluebirds' range across the northwestern United States over the last 40 years (Duckworth and Badyaev 2007).

Here, we use a complex multigenerational pedigree of individually marked western bluebirds to assess genetic integration between components of dispersal strategies and to determine whether the link between these components is the result of their common dependence on genetic or environmental factors. If selection for coexpression of these traits has been strong and consistent across generations, then we predicted that the positive phenotypic correlation between these behaviors would be underlain by a genetic correlation. We use long-term data of lifetime reproductive success and repeated measures of aggression to determine whether, once an individual's dispersal strategy is set, current social environment, breeding stage, or age influence the expression of aggression and whether there are fitness consequences of flexibility of aggression. On the one hand, we might expect selection to act against high flexibility of aggression if this enables closer integration of the two behavioral traits. On the other hand, small changes in aggression within each dispersal strategy may be adaptive if they allow individuals to respond appropriately to current environmental variation.

Methods

STUDY SYSTEM

Western bluebirds are obligate secondary cavity-nesters that depend on nest cavities to reproduce, but cannot excavate their own. Nest cavities are a limited resource (Brawn and Balda 1988; Holt and Martin 1997) leading to intense competition with both conspecifics and other cavity-nesting species (Guinan et al. 2000).

In addition to nest cavities, bluebirds aggressively defend large breeding territories in which they forage for themselves and their offspring. Data used in this study were collected in a population of western bluebirds breeding in the eastern part of Lolo National Forest in western Montana (see Duckworth 2006a for a detailed description of the study site). Western bluebirds colonized this location ca. 15 years ago (Duckworth and Badyaev 2007) and the population size varies around 80 breeding adults per year.

MEASUREMENT OF DISPERSAL AND AGGRESSION

The 600-hectare study site has a discrete boundary as it is surrounded by habitat unsuitable for bluebird breeding—by dense forest to the west and south and by housing subdivisions to the east and north (Duckworth 2006a). Western bluebirds display high site fidelity as adults and the decision to disperse is made during the juvenile stage (Guinan et al. 2000). We categorized individuals as philopatric when they were banded as nestlings at the study site and settled at the study site as a breeding adult. Dispersers were identified as either adults breeding at the site for the first time that dispersed from another population or offspring that were banded at the study site as nestlings but were recaptured as breeders outside the study site (~12% of resighted sons). The latter category were identified through extensive searches of bluebird trails in the 30-km radius of the study area as well as by records of dispersing individuals in an area of ca. 300-km radius around the study site from members of Mountain Bluebird trails, a nonprofit organization whose members maintain and monitor nest box trails throughout Montana, Idaho, and Wyoming.

Following a previously established protocol (Duckworth 2006b), aggression was measured by simulating a territorial intrusion of an interspecific competitor, the tree swallow (*Tachycineta bicolor*). The tree swallow is a widespread nest-site competitor that co-occurs with western bluebirds throughout the northern part of their range. To prevent pseudoreplication during aggression trials, different tree swallows were used each day. Briefly, to simulate territorial intrusions, adults birds were presented with a live tree swallow in a wire cage placed on the nest box. Presenting a common heterospecific competitor controls for prior interactions of conspecifics and objectively and repeatedly assesses aggressive response (Duckworth 2006b). We recorded the number of times an individual attacked, flew by, or hovered near the live model during a 2-min trial. These behaviors were summarized into an aggression score that varied from 1 to 6 with 1 indicating the least aggressive response and a 6 indicating the most aggressive response. Specifically, scores were assigned according to the following scale: 1 = no aggressive behaviors, 2 = hovering or flying by one to five times and 0 attacks, 3 = hovering or flying by more than five times and 0 attacks, 4 = one to five attacks, 5 = six to nine attacks, and 6 = 10 or more attacks. Measurement of aggression using this method is closely linked to territory acquisi-

tion (with more aggressive males gaining larger and higher quality territories) and is repeatable within individuals (Duckworth 2006a,b).

Aggressive behavior of all adults was measured during one of three breeding stages: the incubation stage for all individuals or, for a subset of individuals, the nest building, and/or nestling periods ($n = 60$ males and 37 females). For individuals that returned to breed at the study site in multiple years (min = 2 years, max = 6 years, $n = 49$ males and 44 females), aggression was measured across years as well. Adults were aged as either SY (second year individuals in their first year of breeding) or ASY (after second year) based on banding data and on the molt progress of greater secondary wing coverts (Shizuka and Dickinson 2005). Individuals born before 2001 were grouped as cohort 1 and all other cohorts corresponded sequentially to birth year (e.g., individuals in cohort 2 were born in 2001, cohort 3 were born in 2002, etc.). We acquired 459 measurements of aggression of adults in the study population. We used repeated measures of the same individuals to estimate a permanent environment effect using a quantitative genetic model (see below).

PEDIGREE CONSTRUCTION

Starting in 2001, all nestlings and breeding adults were individually marked with color bands each year, and pair affiliations of breeding adults were determined through extensive behavioral observations. About 38% of nests have extra-pair offspring, ~15% of offspring are extra-pair and divorce among pair mates is common across years (Duckworth 2006b) leading to “natural cross-fostering” which enabled us to more accurately estimate genetic parameters. The pedigree contained both maternal and paternal half-siblings (due to both extra-pair paternity and divorce) as well as full- and half-siblings reared in different nests (within and across breeding seasons). The pedigree structure was further verified by genotyping nestlings and adults at four polymorphic microsatellite loci (see Duckworth 2006b for details on the laboratory protocol). Parentage was assessed for each nest by comparing genotypes of offspring and the attending adults. Sampling of males was nearly complete—all males were sampled from 2003 to 2008 and only 1 and 3 males were not be captured in 2001 and 2002, respectively. All females matched their offspring’s genotype and paternity for the attending male was excluded if one or more loci did not match. Extra-pair offspring were assigned to a sire if their genotypes matched completely and there was no other male in the population with a matching genotype. Two offspring matched three potential fathers and therefore, paternal links were omitted for these individuals. Overall, 98% of extra-pair offspring were assigned to a sire. The pedigree contained 873 individuals with a maximum depth of six generations and dispersal status and/or aggression scores (see below) were available for 274 breeding adults.

ESTIMATION OF VARIANCE COMPONENTS

We estimated variance components and breeding values using a pedigree-based restricted maximum-likelihood mixed model or animal model (Lynch and Walsh 1998; Kruuk 2004). Diagnostics for the model confirmed an assumption of normal errors for aggression. We used a generalized linear model with a binomial error structure to determine whether sex and cohort affected dispersal status. For aggression, we used a mixed model with individual identity included as a random factor to examine whether sex, age, cohort, and mate's response affected aggression. Significant factors were included as fixed effects in the animal model.

Components of variance were estimated using ASReml (VSN International: www.vsn-intl.com). We first used a univariate model of the repeated measures of aggression to determine whether there was a significant component of variance due to permanent environment effects, namely persistent differences among individuals not already attributed to V_A such as the effects of environmental conditions experienced during early development (Kruuk and Hadfield 2007). For the univariate model using repeated measures, the total phenotypic variance (V_P) was partitioned into the additive genetic (V_A), permanent environment (V_{PE}), and residual (V_R) components. Genetic variances and covariances of both traits were calculated from a bivariate model of dispersal and aggression, using a single measure of individual aggression defined as the average across any repeated measures. For the bivariate model, V_P was partitioned into V_A and V_R alone. We also fitted a maternal effect, an effect for social father, and a nest effect; however, these were not significant (all $P > 0.85$) and were not included in the final model.

Aggression was treated as a single trait common to both sexes (with a fixed effect of sex), as allowing sex-specific variance components did not improve the model. For the bivariate analysis, we included aggression scores of both sexes but dispersal status of males only because dispersal behavior of females differed substantially from males, showing markedly less phenotypic variance. Whereas 44.2% of males were philopatric, only 7.4% of females returned to their natal population to breed and all other females had dispersed into the area and so, because their parents were unknown, were effectively part of the base population. Dispersal was considered a "threshold trait," which assumes that the two dispersal options are underlain by a continuous distribution (Roff 1997; Lynch and Walsh 1998). We transformed heritability of dispersal on the observed scale (h_o^2) to an assumed underlying continuous normally distributed scale using the equation $h_c^2 = h_o^2 p(1-p)/z^2$, where p is the incidence in the population and z is the height of the standardized normal distribution for this incidence (Lynch and Walsh 1998). Standard errors were scaled using the same equation. We similarly transformed the observed phenotypic correlation between dispersal and ag-

gression (r_o) using the equation $r_c = r_o \sqrt{p(1-p)/z^2}$ (following Brotherstone et al. 1990). Genetic correlations on the underlying scale are not expected to differ from the observed scale (ibid.). We calculated coefficients of additive genetic variation for aggression (CV_A) using the equation $100\sqrt{V_A/\bar{X}}$ where \bar{X} is the trait mean (Houle 1991). The significance of variance and covariance components were tested using a likelihood-ratio test (Pinheiro and Bates 2000).

SELECTION ON FLEXIBILITY OF AGGRESSION

In western bluebirds, the most important social environment during breeding is an individual's mate and because we found a strong correlation between mates in their aggressive behavior (see below), we investigated whether flexible adjustment of aggression could account for this correlation. We capitalized on the high incidence of mate switching among individuals between years due to divorce or mortality of their previous mate to determine whether aggression of mates was correlated due to either assortative pairing (i.e., neither males nor females modify their aggressive phenotype and similarity in aggression occurs because individuals choose partners similar to them in aggressive phenotype) or flexible adjustment of aggression by either sex to match their partners' aggression. To test for flexible adjustment of aggression in relation to their mate, we calculated the absolute value of mean change in aggression of an individual across sequential mates. For example, if an individual had three different mates, we calculated the absolute value of the difference in mean aggression while paired with the first mate from mean aggression while paired with the second as well as the absolute value of the difference in mean aggression while paired with the second mate from mean aggression while paired with the third and used the average of these for analyses. We used a general linear model to relate these changes in aggression within individuals to sex, the mean difference in aggression of their mates, and the interaction between sex and difference in mate's aggression scores.

To determine whether adjustment of aggression was adaptive, we calculated selection on flexibility of aggression by estimating the selection gradient from a regression of relative fitness on the absolute value of an individual's average change in aggression across mates. Lifetime fitness (defined as total number of fledged offspring) was square-root transformed, where appropriate, to improve normality. To determine whether there were fitness consequences of matching a mate's aggression, we related relative fitness to a "mismatch" score, defined as the absolute value of the difference in pair mates' aggression scores for each year and then averaged across years such that individuals who generally had very similar levels of aggression to their mate had mismatch values close to zero, whereas those with very different aggressive levels had higher values.

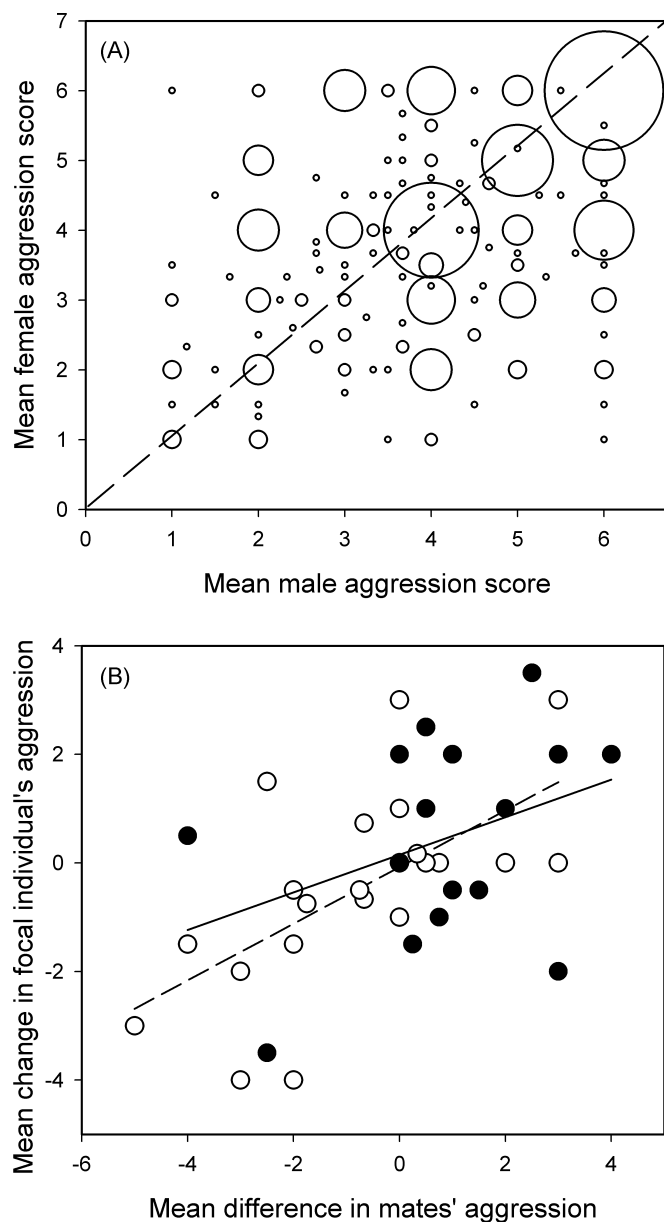


Figure 1. (A) Correlated aggressive response of mated individuals in western bluebirds. Repeated measures of individuals were averaged. Points along dashed line indicate mated pairs with identical responses. Size of circles indicate the number of overlapping datapoints with the smallest size indicating a single point and the largest indicating six overlapping points. (B) Individuals flexibly changed their aggressive response to match their mate's behavior. Black circles and solid line indicate females and white circles and dashed line indicate males.

Results

HERITABILITY, GENETIC CORRELATION, AND VARIANCE COMPONENTS

Aggression was affected by both an individual's sex (mean \pm SE aggression score of females: 3.76 ± 0.13 vs. males: 4.27 ± 0.11 ;

$F_{1,188} = 19.92$, $P < 0.001$) and their mate's aggression score such that there was a positive correlation between aggression of mated individuals ($F_{1,188} = 105.35$, $r = 0.42$, $P < 0.001$; Fig. 1A). Aggression did not differ between age classes ($F_{1,188} = 0.72$, $P = 0.40$), breeding stages ($F_{2,188} = 0.29$, $P = 0.74$), or cohorts ($F_{7,188} = 1.63$, $P = 0.13$). Dispersal behavior was strongly sex-biased (among breeders 7.4% of females vs. 44.2% of males were philopatric, $\chi^2 = 58.26$, $P < 0.001$) and the percent of breeding males categorized as dispersers differed among cohorts ($\chi^2 = 24.40$, $P = 0.001$). Estimates of the heritability of aggression were significant in both the univariate (using repeated measures; $h^2 = 0.34 \pm 0.13$, $P < 0.01$) and the bivariate analyses (using averaged aggression scores; $h^2 = 0.52 \pm 0.17$, $P < 0.01$; Table 1) but there was no evidence of a significant component of variance due to permanent environment effects (Table 1). Omitting the permanent environment effect from the univariate model increased estimates of heritability of aggression to 0.42 ± 0.06 . Bivariate analysis indicated that there was significant additive genetic variance for dispersal behavior and that aggression and dispersal were positively genetically correlated ($r_g = 0.55 \pm 0.24$, $P = 0.048$); however, there was no significant environmental correlation between these behaviors (Table 1).

SELECTION ON FLEXIBILITY OF AGGRESSION

Individuals modified their level of aggression to match their mate's aggression: changes in aggression within individuals closely corresponded to differences in aggression between consecutive mates ($F_{1,54} = 24.95$, $r = 0.56$, $P < 0.001$; Fig. 1B). There was no difference among the sexes in the extent to which they modified their aggression between consecutive partners ($F_{1,54} = 1.75$, $P = 0.19$) and both sexes responded similarly strongly to differences in the behavior of consecutive mates (interaction between sex and difference in mate's behavior: $F_{1,54} = 1.50$, $P = 0.23$). Selection on flexibility of aggression was weak and not significant—there were no significant fitness consequences for either matching a mate's behavior ($F_{1,211} = 0.79$, $b = 0.06$, $P = 0.38$; Fig. 2A) or for modifying aggression between consecutive mates ($F_{1,41} = 0.70$, $b = -0.13$, $P = 0.41$; Fig. 2B).

Discussion

Discrete behavioral strategies comprise a suite of traits that are integrated in their expression. How such complex behavioral phenotypes evolve remains an important problem in evolution because behavioral traits should be under strong selection for both greater context-dependency to enable a rapid response to changing environments (DeWitt et al. 1998) and, at the same time, for greater integration with other traits to ensure that the organism as a whole functions properly.

Table 1. Components of variance (V_A , additive genetic variance; V_{PE} , permanent environment effect variance; and V_R , residual variance), heritability (h^2), coefficient of additive genetic variation (CV_A), and correlations (r) for aggression and dispersal using univariate and bivariate models. Standard errors are in parentheses. * $P < 0.05$, ** $P < 0.01$, P values estimated from likelihood-ratio tests.

Model	Trait	r	Mean	V_A	V_{PE}	V_R	h^2	CV_A
Univariate	Aggression ¹		3.88 (0.09)	0.67 (0.28)	0.17 (0.25)	1.15 (0.11)	0.34 (0.13)**	21.10
Bivariate	Aggression ²		4.02 (0.09)	0.89 (0.32)		0.82 (0.28)	0.52 (0.17)**	23.47
	Dispersal ³		0.57 (0.04)	0.13 (0.05)		0.08 (0.04)	0.60 (0.20)**	63.25
	Dispersal ⁴						0.95 (0.31)**	
	Phenotypic ³	0.22 (0.09)*						
	Phenotypic ⁴	0.28 (0.11)*						
	Genetic	0.55 (0.24)*						
	Environmental	-0.21 (0.29)						

¹Repeated measures.

²Average score.

³Estimated on the observed scale.

⁴Estimated on the underlying scale.

Our study of the genetics of dispersal and colonization behavior of western bluebirds produced three main results. First, we found evidence of genetic integration between heritable aggression and dispersal behavior—dispersive fathers were more likely to produce aggressive offspring and philopatric fathers were more likely to produce nonaggressive offspring. Second, we found that although aggression is repeatable (Duckworth 2006b; Duckworth and Badyaev 2007), individuals modified their aggressive behavior within each strategy to match their current mate's aggressive response. Third, we found no strong fitness consequences of either matching a mate's aggression or the extent to which individuals modified their own aggression. Taken together and in conjunction with previous work in this system, these results support the idea that consistent selection for coexpression of aggression and dispersal has led to their genetic integration. However, these results raise several questions. How and why is genetic integration among these behaviors maintained despite flexibility in the expression of aggression? Why do individuals match their mates' aggressive behavior? Finally, what are the proximate developmental mechanisms underlying the link between these behaviors?

The juvenile dispersal decision is a threshold trait (Roff 1994) and is expressed only once as a decision to either leave from or remain in the natal population to breed; whereas aggressive behavior is expressed repeatedly throughout adulthood. How can these behaviors be strongly genetically integrated (Table 1) despite such distinct time scales for their expression? One possibility is that integration occurs during a critical period early in life when the general level of expression of both traits is determined. Under this scenario, common developmental mechanisms influence both dispersal propensity and the expression of aggression and although aggression retains some limited flexibility, its expression remains relatively consistent within individuals (e.g.,

Supporting Fig. S1). This model is supported by the observed repeatability of aggression, which indicates that there is some limit to flexibility of aggression (Duckworth 2006b; Duckworth and Badyaev 2007) as well as by the fact that integration between these behaviors is underlain by a genetic rather than environmental correlation indicating that phenotypic coexpression of these traits is stable across multiple generations. Thus, these results suggest that integration of aggression and dispersal is coordinated by shared genes but the actual strategy that any given individual takes is influenced by both genetic and environmental variation.

We found that aggressive behavior varied in relation to an individual's current social environment such that individuals matched the aggressive behavior of their mates. However, neither matching a mate's behavior nor the extent to which individuals adjusted their own behavior had detectable fitness consequences (Fig 1). Indeed, although not significant, measurement of selection on flexibility of aggression in this study was negative suggesting that, rather than being beneficial, there may be a small fitness cost of expressing flexibility in aggressive response. Why then do individuals adjust their behavior to match their mate's behavior? It may be that the tendency of individuals to adjust their own behavior in relation to their mate's is a consequence of the general propensity of mated pairs to coordinate their behavior during breeding. An alternative explanation is that mates are modifying their behavior, not in response to each other, but instead, in response to some third unmeasured factor. Experimental manipulation of aggression will be needed to rule out this possibility. In either case, given the weak fitness consequences documented in this study, modifying aggression in response to a mate's aggressive behavior is not likely to be of strong functional importance in this system.

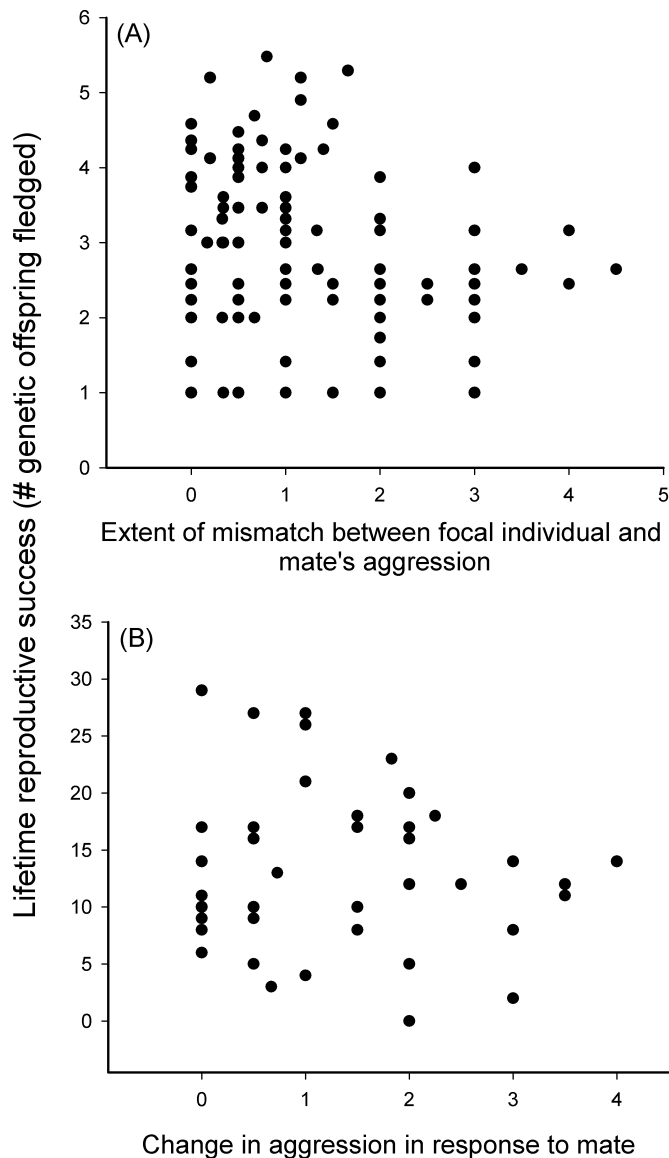


Figure 2. Selection on flexibility of aggression. Relationship between lifetime reproductive success and (A) extent of match to mate's aggression (calculated as absolute value of average focal individual minus mates' aggression score) and (B) change in aggression in response to difference in mates' aggression (calculated as absolute value of mean change in aggression across consecutive mates). Lifetime reproductive success is square-root transformed in A to normalize data.

An obvious question stemming from these results is whether the genetic correlation between aggression and dispersal is due to linkage disequilibrium or pleiotropy. Although strong selection for coexpression can produce a genetic correlation through linkage disequilibrium over the short term, over the long term, this selection is expected to favor pleiotropy (Lande 1980; Cheverud 1996; Wagner 1996). So the question becomes whether the genetic link between these traits has arisen in this species' recent history, perhaps in association with a range expansion that favors integra-

tion of these traits (Duckworth and Badyaev 2007) or whether it has evolved over long evolutionary time outside the context of the current range expansion? Breeding experiments and/or studies of the development of these behaviors are necessary to distinguish between these alternative genetic mechanisms; however, several observations suggest that selection for integration of dispersal and aggression precedes this species' recent range expansion. First, the distinct dispersal strategies of males occur in ancestral populations at similar frequencies to those observed in recently the established populations (Dickinson and Akre 1998; Charmantier et al. 2007; Duckworth and Badyaev 2007) suggesting that they are not of recent origin. Second, experimental work has shown that the link between aggression and dispersal is not limited to the unique circumstances of the range expansion, but instead, occurs whenever western bluebirds colonize new areas, even when these areas are in the interior of the range (Duckworth 2008). Third, the ecology of western bluebirds suggests that distinct dispersal strategies were likely adaptive historically. Before the placement of nest boxes, western bluebirds depended on cavities in dead trees that were often concentrated in recently burned forests (Hutto 1994). These areas can have exceptionally high densities of natural nest cavities but are stable for only 5–10 bluebird generations creating a mosaic of old and new habitat patches. Thus, evolution of distinct dispersal strategies in this species may be an adaptation to this historical resource distribution whereby some males benefit from colonizing newly formed patches whereas others benefit from inheritance of nest cavities in older patches. As evidence for this, the creation of experimental populations interior to the range front has shown that aggressive males that disperse have higher fitness when colonizing new habitat patches whereas nonaggressive philopatric males have the highest fitness in older patches (Duckworth 2008). Finally, the decision to stay or disperse is simultaneously the choice of either an old familiar habitat or a new unfamiliar habitat making the dispersal decision a form of niche construction as it creates the functional context in which the association of these two traits is adaptive (Odling-Smee et al. 2003). Therefore, in this case, the phenotype itself provides the means for maintaining consistent selection on the coexpression of these traits. Taken together, these observations suggest that the link between these traits was likely adaptive historically and thus, that selection for coexpression of these traits is not of recent origin in response to this species range expansion, but instead has been consistent over evolutionary time.

Evolution of pleiotropy among behavioral traits can occur through the coexpression of genes that affect the formation of shared neural pathways, the distribution of hormone receptors, and the development of the endocrine controls of behavioral components of an integrated module (e.g., Rhen and Crews 2002). Recent work in diverse taxa shows that dispersal behavior may covary with underlying temperament traits, such as boldness or

exploratory behavior (Dingemanse et al. 2003; Rehage and Sih 2004) that are commonly correlated with aggression (Huntingford 1976; Wilson et al. 1994; Rehage and Sih 2004; Bell 2005). This raises the possibility that variation in temperament observed in diverse taxa is a consequence of selection for functional integration of distinct phenotypic components and also suggests that similar developmental mechanisms that underlie variation in temperament traits may ultimately link aggression and dispersal (Bakker 1994; Sih et al. 2004; van Oers et al. 2004; 2005).

In this population, nearly all (92.9%) females dispersed and thus, we did not know the parents of most breeding females. This scenario precluded quantitative genetic analysis of any female-specific trait. However, there were no sex differences in the genetic architecture of aggression, and because of the lack of phenotypic variance in dispersal of females we only considered male dispersal in the analyses. Moreover, as is the case for all studies of heritability of dispersal in wild populations (van Noordwijk 1984), measurement of dispersal in offspring is necessarily incomplete as it is likely that some dispersing offspring were not recaptured. However, our use of a network of trail monitors throughout a ca. 300-km radius surrounding the study area meant that our search for dispersers covered a large region making it unlikely to be strongly biased with respect to dispersal distance.

The link between dispersal and aggression in this system is analogous in several ways to distinct dispersal polymorphisms described in many insect and plant species (Sorensen 1978; Harrison 1980; Zera and Denno 1997). First, in these species, dispersal is linked to traits that increase either mobility or colonization ability. In western bluebirds, high aggression increases colonization ability by enabling males to successfully acquire territories in new habitat in which they face competition from both con- and heterospecifics (Duckworth 2006a). Second, in species with distinct dispersal strategies, dispersal is not just integrated with functional traits (e.g., winged versus nonwinged insect morphs), but is also linked to distinct life-history strategies due to a trade-off between investment in dispersal-related traits and fecundity (Roff 1984). In western bluebirds, aggression is costly in terms of fecundity because highly aggressive males invest less in parental care (Duckworth 2006b) suggesting that the link between aggression and dispersal is but one axis of a suite of interrelated behaviors that have evolved as a consequence of the fundamental life-history trade-offs associated with dispersal. Finally, species that display distinct dispersal strategies often depend on ephemeral habitat and variation in dispersal strategy is maintained in these systems through strong spatial and temporal variation in habitat availability which leads to spatial and temporal variation in selection on dispersal strategy (Roff 1986; Johnson and Gaines 1990; McPeck and Holt 1992). In western bluebirds, because aggressive males benefit by colonizing newly formed habitat patches whereas nonaggressive males benefit from inheritance of nest

cavities in older patches, selection on dispersal phenotypes varies both spatially and temporally in this species (Duckworth 2008). Such temporal and spatial variation in selection pressures can not only maintain variation in these behaviors, but on a regional scale, can also produce correlational selection for their coexpression. Taken together, the similarities between distinct behavioral strategies of western bluebirds and dispersal polymorphisms of other taxa suggest that the trade-offs associated with dispersal are universal and can lead to similar evolutionary outcomes in a wide range of organisms.

Selection for functional integration of behavior and resulting genetic integration may be a common mechanism for the evolution of behavioral strategies. Yet, the underlying genetics of behavioral variation are often overlooked in studies of the adaptive significance of behavioral variation (Reeve and Sherman 1993) even though, over the short term, genetic correlations among behaviors should bias or constrain the response to selection, potentially leading to maladaptive expression of behaviors under changing environmental conditions. Thus, ignoring the underlying developmental causes (both genetic and environmental) of behavioral variation may lead to misleading interpretations of its adaptive significance (Arnold 1988; Crespi 2000; Sih et al. 2004). Long-term integrative studies of marked populations and the recent application of animal models to natural populations are likely to provide important new insights into the genetics of adaptive behavioral strategies in wild populations. Although suites of integrated behaviors with their complexity and context-dependent expression have been notoriously difficult to study within a rigorous evolutionary biology framework, quantitative genetic analysis of behaviors is likely to be particularly rewarding as it provides important insights into evolutionary, functional, and developmental biology of one of the most visible and important aspects of the phenotype—its behavior.

ACKNOWLEDGMENTS

We thank A. Badyaev, T. Price, B. Hill, A. Wilson, J. Hadfield, and S. Brotherstone for useful discussions and comments on previous version of the manuscript. We thank the USFS for permission to work at the Lolo National Forest and members of Mountain Bluebird Trails, in particular, E. Davis and A. Osellame for contributing information on recaptured birds and N. Clyde and T. Dockter for permission to monitor their nest box trails. Funding was provided to RAD by the National Science Foundation and to LEBK by the Royal Society, London.

LITERATURE CITED

- Arnold, S. J. 1988. Behavior, energy and fitness. *Am. Zool.* 28:815–827.
 Atchley, W. R. 1984. Ontogeny, timing of development, and genetic variance-covariance structure. *Am. Nat.* 123:519–540.
 Atchley, W. R., and B. K. Hall. 1991. A model for the development and evolution of complex morphological structures. *Biol. Rev.* 66:101–157.
 Atchley, W. R., S. Xu, and C. Vogl. 1994. Developmental quantitative genetic models of evolutionary change. *Develop. Gen.* 15:92–103.

- Badyaev, A. V. 2002. Growing apart: ontogenetic perspective on the evolution of sexual size dimorphism. *Trends Ecol. Evol.* 17:369–378.
- . 2004. Integration and modularity in the evolution of sexual ornaments: an overlooked perspective. Pp. 50–79 in M. Pigliucci and K. Preston, eds. *Phenotypic integration: the evolutionary biology of complex phenotypes*. Oxford Univ. Press, Oxford.
- Badyaev, A. V., and G. E. Hill. 2002. Paternal care as a conditional strategy: distinct reproductive tactics associated with elaboration of plumage ornamentation in the house finch. *Behav. Ecol.* 13:591–597.
- Bakker, T. C. M. 1994. Genetic correlations and the control of behavior, exemplified by aggressiveness in sticklebacks. *Adv. Study Behav.* 23:135–171.
- Bell, A. M. 2005. Behavioral differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). *J. Evol. Biol.* 18:464–473.
- Brawn, J. D., and R. P. Balda. 1988. Population biology of cavity nesters in northern Arizona: do nest sites limit breeding densities? *Condor* 90:61–71.
- Brotherstone, S., M. G. McManus, and W. G. Hill. 1990. Estimation of genetic parameters for linear and miscellaneous type traits in Holstein-Friesian dairy cattle. *Livest. Prod. Sci.* 26:177–192.
- Charmantier, A., A. J. Keyser, and D. E. L. Promislow. 2007. First evidence of heritable variation in cooperative behaviour. *Proc. R. Soc. Lond. B* 274:1757–1761.
- Cheverud, J. M. 1982. Phenotypic, genetic, and environmental morphological integration in the cranium. *Evolution* 36:499–516.
- . 1996. Developmental integration and the evolution of pleiotropy. *Am. Zool.* 36:44–50.
- Crespi, B. J. 2000. The evolution of maladaptation. *Heredity* 84:623–629.
- DeWitt, T. J., A. Sih, and D. S. Wilson. 1998. Costs and limits to phenotypic plasticity. *Trends Ecol. Evol.* 13:77–81.
- Dickinson, J. L., and J. J. Akre. 1998. Extrapair paternity, inclusive fitness, and within-group benefits of helping in western bluebirds. *Mol. Ecol.* 7:95–105.
- Dingemanse, N. J., C. Both, A. J. van Noordwijk, A. L. Rutten, and P. J. Drent. 2003. Natal dispersal and personalities in great tits (*Parus major*). *Proc. R. Soc. Lond. B* 270:741–747.
- Duckworth, R. A. 2006a. Aggressive behavior affects selection on morphology by determining the environment of breeding in a passerine bird. *Proc. R. Soc. Lond. B* 273:1789–1795.
- . 2006b. Behavioral correlations across reproductive contexts provide a mechanism for a cost of aggression. *Behav. Ecol.* 17:1011–1019.
- . 2008. Adaptive dispersal strategies and the colonization of a novel environment. *Am. Nat.* 172:S4–S17.
- . 2009. The role of behavior in evolution: a search for mechanism. *Evol. Ecol.* doi: 10.1007/s10682-008-9252-6.
- Duckworth, R. A., and A. V. Badyaev. 2007. Coupling of aggression and dispersal facilitates the rapid range expansion of a passerine bird. *Proc. Natl. Acad. Sci. USA* 104:15017–15022.
- Emlen, D. J. 1997. Alternative mating tactics and male dimorphism in the horned beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Behav. Ecol. Sociobiol.* 41:355–341.
- Fairbairn, D. J., and D. A. Roff. 1990. Genetic correlations among traits determining migratory tendencies in the sand cricket, *Gryllus firmus*. *Evolution* 44:1787–1795.
- Gaines, M. S., and L. R. McClenagham. 1980. Dispersal in small mammals. *Annu. Rev. Ecol. Syst.* 11:163–196.
- Guinan, J. A., P. A. Gowaty, and E. K. Eltzroth. 2000. Western Bluebird (*Sialia mexicana*). *The Birds of North America*, No. 510. Birds of North America, Inc., Philadelphia, PA.
- Harrison, R. G. 1980. Dispersal polymorphisms in insects. *Annu. Rev. Ecol. Syst.* 11:95–118.
- Holt, R., and K. Martin. 1997. Landscape modification and patch selection: the demography of two secondary cavity nesters colonizing clearcuts. *Auk* 114:443–455.
- Houle, D. 1991. Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. *Evolution* 45:630–648.
- Huntingford, F. A. 1976. The relationship between anti-predator behavior and aggression among conspecifics in the three-spined stickleback, *Gasterosteus aculeatus*. *Anim. Behav.* 24:245–260.
- Hutto, R. L. 1994. Composition of bird communities following stand-replacement fires in northern rocky mountain (U.S.A.) conifer forests. *Conserv. Biol.* 9:1041–1058.
- Johnson, M. L., and M. S. Gaines. 1990. Evolution of dispersal: theoretical models and empirical tests using birds and mammals. *Annu. Rev. Ecol. Syst.* 21:449–480.
- Kruuk, L. B. 2004. Estimating genetic parameters in natural populations using the ‘animal model’. *Philos. Trans. R. Soc. Lond. B* 359:873–890.
- Kruuk, L. E. B., and J. D. Hadfield. 2007. How to separate genetic and environmental causes of similarity between relatives. *J. Evol. Biol.* 20:1890–1903.
- Lande, R. 1979. Quantitative genetic analysis of multi-variate evolution, applied to brain : body size allometry. *Evolution* 33:402–416.
- . 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* 34:292–305.
- Lynch, M., and B. Walsh. 1998. *Genetics and analysis of quantitative traits*. Sinauer Associates, Sunderland, MA.
- McPeck, M. A., and R. D. Holt. 1992. The evolution of dispersal in spatially and temporally varying environments. *Am. Nat.* 140:1010–1027.
- Odling-Smee, F. J., K. N. Laland, and M. W. Feldman. 2003. *Niche Construction: the neglected process in evolution*. Princeton Univ. Press, Princeton, NJ.
- Pinheiro, J. C., and D. M. Bates. 2000. *Mixed-effects models in S and S-Plus*. Springer, New York.
- Reeve, H. K., and P. W. Sherman. 1993. *Adaptation and the goals of evolutionary research*. *Q. Rev. Biol.* 68:1–32.
- Rehage, J. S., and A. Sih. 2004. Dispersal behavior, boldness, and the link to invasiveness: a comparison of four *Gambusia* species. *Biol. Invas.* 6:379–391.
- Rhen, T., and D. Crews. 2002. Variation in reproductive behavior within a sex: neural systems and endocrine activation. *J. Neuroendocrinol.* 14:517–531.
- Riska, B. 1986. Some models for development, growth, and morphometric correlation. *Evolution* 40:1303–1311.
- . 1989. Composite traits, selection response and evolution. *Evolution* 43:1172–1191.
- Roff, D. A. 1984. The cost of being able to fly: a study of wing polymorphism in two species of crickets. *Oecologia* 63:30–37.
- . 1986. The evolution of wing dimorphism in insects. *Evolution* 40:1009–1020.
- . 1994. Habitat persistence and the evolution of wing dimorphism in insects. *Am. Nat.* 144:772–798.
- . 1997. *Evolutionary quantitative genetics*. Longman, New York.
- Roff, D. A., and D. J. Fairbairn. 2001. The genetic basis of dispersal and migration and its consequences for the evolution of correlated traits. Pp. 191–202 in J. Clobert, E. Danchin, A. A. Dhondt and J. D. Nichols, eds. *Dispersal*. Oxford Univ. Press, New York.
- Schlichting, C. D., and M. Pigliucci. 1998. *Phenotypic evolution. A reaction norm approach*. Sinauer Associates, Sunderland, MA.
- Shizuka, D., and J. L. Dickinson. 2005. Using molt limits to age Western Bluebirds. *J. Field Ornithol.* 76:193–196.
- Shuster, S. M., and M. J. Wade. 2003. *Mating systems and strategies*. Princeton Univ. Press, Princeton, NJ.

- Sih, A., A. Bell, J. C. Johnson. 2004. Behavioral syndromes: an ecological and evolutionary overview. *Trends Ecol. Evol.* 19:372–378.
- Skjelseth, S., T. H. Ringsby, J. Tufto, H. Jensen, and B. E. Saether. 2007. Dispersal of introduced house sparrows *Passer domesticus*: an experiment. *Proc. R. Soc. Lond. B* 274:1763–1771.
- Toonen, R. J., and J. R. Pawlik. 2001. Foundations of gregariousness: a dispersal polymorphism among the planktonic larvae of a marine invertebrate. *Evolution* 55:2439–2454.
- Sgrò, C. M., and A. A. Hoffman. 2004. Genetic correlations, tradeoffs and environmental variation. *Heredity* 93:241–248.
- Sorensen, A. E. 1978. Somatic polymorphism and seed dispersal. *Nature* 276:174–176.
- Tuttle, E. M. 2002. Alternative reproductive strategies in the white-crowned sparrow: behavioral and genetic evidence. *Behav. Ecol.* 14:425–432.
- van Noordwijk, A. J. 1984. Problems in the analysis of dispersal and a critique of its 'heritability' in the great tit. *J. Anim. Ecol.* 53:533–544.
- van Oers, K., G. de Jong, P. J. Drent, and A. J. van Noordwijk. 2004. A genetic analysis of avian personality traits: correlated, response to artificial selection. *Behav. Genet.* 34:611–619.
- van Oers, K., G. de Jong, A. J. van Noordwijk, B. Kempenaers, and P. J. Drent. 2005. Contribution of genetics to the study of animal personalities: a review of case studies. *Behavior* 142:1191–1212.
- Wagner, G. P. 1996. Homology, natural kinds and the evolution of modularity. *Am. Zool.* 36:36–43.
- . 2001. *The character concept in evolutionary biology*. Academic Press, San Diego.
- West-Eberhard, M. 2003. *Developmental plasticity and evolution*. Oxford Univ. Press, New York.
- Wilson, D. S., A. B. Clark, K. Coleman, and T. Dentstynne. 1994. Shyness and boldness in humans and other animals. *Trends Ecol. Evol.* 9:442–446.
- Zera, A. J., and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. *Ann. Rev. Entomol.* 42:207–230.

Associate Editor: M. Webster

Supporting Information

The following supporting information is available for this article:

Figure S1. An example of variation in flexibility of aggression across four individuals.

Supporting Information may be found in the online version of this article.

(This link will take you to the article abstract).

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting informations supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.