

## Food, sex and predators: animal personality persists with multidimensional plasticity across complex environments



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The payoffs of an individual's behaviour vary with changing environmental conditions. Animals often modify their behaviours according to those environmental conditions (i.e. plasticity), but also retain consistent individual differences across environmental change (i.e. personality). These patterns of behavioural variation are often measured with respect to a single environmental variable, which raises the question of how individuals respond to change in combinations of environmental variables, and whether individual differences in behaviour persist across change in multiple variables. Furthermore, an individual's amount of plasticity in response to a change in one environmental variable may or may not be repeatable across change in a second environmental variable. To answer these questions, we experimentally manipulated combinations of mating, foraging and predation risk to determine their effect on the antipredator behaviour of male Belding's ground squirrels, *Uroditellus beldingi*. We found that the combination of environmental variables had an interactive effect on antipredator behaviour, but among-individual variation persisted along with within-individual variation in behaviour. The plasticity of squirrels' responses to change in one environmental variable, such as change in antipredator behaviour from high to low predation risk, was repeatable when measured in a mating environment and foraging environment, and vice versa. These results demonstrate patterns of behavioural variation across complex environments such as animals encounter in nature, and point towards the benefit of addressing greater environmental complexity in studies of animal behaviour.

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When animals exhibit consistent individual differences in behaviour over time and across environmental change, we refer to this as displaying personality or a behavioural syndrome (Réale, Reader, Sol, McDougall, & Dingemanse, 2007; Sih, Bell, & Johnson, 2004). This among-individual variance contrasts with individuals' behavioural flexibility in response to environmental change, known as behavioural plasticity (Dingemanse, Kazem, Réale, & Wright, 2010; Pigliucci, 2001; West-Eberhard, 1989). Understanding how personality and plasticity contribute to behavioural variation is a primary endeavour of animal behaviour research (Dingemanse et al., 2010; Sih et al., 2004; Stamps & Groothuis, 2010). A number of studies have found that a wide range of species display personality as well as plasticity to environmental variables such as temperature, experience and predation threat (Biro, Beckmann, & Stamps, 2010; Briffa, Rundle, & Fryer, 2008; Martin & Réale, 2008). These results call for studies of animal personality and

behavioural plasticity addressing greater environmental complexity.

In natural environments, an individual's response to one environmental variable may vary depending on the level of other environmental variables. To date, we know of only one study (Westneat, Hatch, Wetzel, & Ensminger, 2011) that assessed personality alongside the plasticity of a trait in response to change in multiple environmental variables, known as multidimensional plasticity (Westneat, Stewart, & Hatch, 2009). Brood size and nestling age have an interactive effect on parental provisioning behaviour of individual house sparrows, *Passer domesticus*, but there is also substantial among-individual variation of provisioning behaviour across variation in those environmental variables (Westneat et al., 2011). Furthermore, as multiple environmental variables fluctuate over time, a plastic response to one environmental variable, such as temperature, may or may not be repeatable at different levels of a second environmental variable, such as predation threat. In other words, is behavioural plasticity repeatable when a different environmental variable also changes? Here we experimentally manipulated combinations of multiple environmental variables in Belding's ground squirrels, *Uroditellus beldingi*, to answer whether multidimensional plasticity persists with

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animal personality, and whether plastic responses of individuals are consistent. Additionally, we compared results from models incorporating individual environmental variables separately to models incorporating multiple environmental variables and their interaction to highlight potential differences among multidimensional plasticity and traditional, unidimensional approaches to plasticity.

Belding's ground squirrels are social rodents that are preyed upon by aerial and terrestrial predators (Mateo, 2007). They use their burrows as safe refuge, but forage and mate aboveground. Thus, individuals that are less risk averse, or bolder, are more likely to spend time aboveground relative to individuals that are more risk averse, or shyer. The balance between risk and reward outside the burrow creates expectations that squirrels' refuge use should vary as that balance shifts. We expect greater refuge use when predation threat is high versus low. Also, we expect greater refuge use in a foraging context versus a mating context, due to the greater evolutionary payoff of a potential mate over that of a potential meal. However, that prediction is predicated on individuals not being substantially food deprived, which will sometimes be the case in the field. An individual with low nutritional status would be expected to spend less time in the refuge in the foraging context. In a sample of wild-caught, captive male *U. beldingi*, we repeatedly measured refuge use of squirrels experiencing combinations of mating, foraging and predation risk environments to address three hypotheses. First, we hypothesized that the refuge use of squirrels shows multidimensional plasticity, which would be indicated by an interactive effect of environmental variables on refuge use. Second, we hypothesized that squirrels show consistent individual differences in refuge use across the manipulated combinations of environmental variables; that is, a relatively bold individual in one situation (combination of environmental variables) would also be relatively bold in another situation. Significant among-individual variance would support this hypothesis. Finally, we hypothesized that repeated expressions of plasticity to one environmental variable, such as predation risk, will show consistent individual differences across multiple environmental contexts, such as foraging and mating contexts. The field of animal personality aims to describe more comprehensively the behavioural variation of populations, and this experiment will help clarify how behaviours vary across complex, more realistic environments.

## METHODS

### Animals

Belding's ground squirrels are social, diurnal rodents that inhabit the eastern Sierra Nevada and southern Cascade mountain ranges in California and Oregon, U.S.A. (Sherman & Morton, 1984). They hibernate each winter and are active between April and September each year. Their mating season is restricted to the first few weeks of the active season (Sherman & Morton, 1984), and the rest of the active season is primarily devoted to foraging to gain weight for the next winter hibernation. Due to the nature of the experimental manipulation of mating environment (see below), and the remarkably short oestrus of female squirrels (~6 h/year; Sherman & Morton, 1984), we only tested male *U. beldingi*. We trapped males from Rock Creek Canyon, CA, housed them individually in plastic cages (38 × 33 × 11 cm; solid sides and bottom, wire lid) with pine bedding and dry grass, and provided them with five pieces of Mouse Diet 5015 (LabDiet, Richmond, IN, U.S.A.) and water ad libitum. We maintained a 13:11 h light:dark cycle to mimic the natural cycle, and maintained temperature using a heater at night and a combination of fans and/or an air conditioner during the day.

In 2009, average daily temperature ( $\pm$ SD) at 1000 hours was  $55.4 \pm 2.6$  °F ( $12.4 \pm 1.4$  °C). After 2009, we switched to a thermometer that recorded daily minimum and maximum temperatures, which averaged  $56.7 \pm 2.0$  °F ( $13.7 \pm 1.1$  °C) and  $72.7 \pm 5.6$  °F ( $22.6 \pm 1.7$  °C). We tested males over the course of a few weeks during the mating season, giving roughly 1 week for acclimation to captivity before testing and 1 week between test repetitions. Tests occurred before or within a few days of our last observed mating in the field. Males had descended and pigmented testes, which indicates sexual capability. We collected data between 2009 and 2011, testing 27 males across the manipulated environments, and an additional 11 males to control for potential order effects in the experimental design (see below). After testing, we released the squirrels at their site of capture. During trapping we took a number of measures to minimize stress. We checked traps every 10–15 min, but more regularly if weather conditions were less than ideal. After an individual was captured, we placed it in the sun or shade as appropriate for the temperature, and provided a small amount of peanut butter as food until transport to the animal quarters. Institutional Animal Care and Use Committees (IACUC) at University of Chicago (protocol no. 71255) and University of California at Santa Barbara (protocol no. 5-03-532) approved this study, which adhered to standards set forth by the ASAB/ABS Guidelines for the Use of Animals in Research and by the U.S. National Institutes of Health for animal research. We had permits from California Fish & Game and U.S. Forest Service.

### Test of Refuge Use

We measured antipredator behaviour of *U. beldingi* in a refuge use test. We focused on refuge use as an antipredator behaviour because it capitalizes on the balance between costs of differing predation risk and differing benefits of foraging and mating. The test consisted of a 122 × 122 × 61 cm arena built of wood, covered with a wire top and lined with an acrylic floor to facilitate cleaning and minimize residual squirrel odours across tests. An artificial burrow system in the centre of the arena provided a refuge similar to burrows in nature. The interconnected burrow system had three openings to facilitate access from anywhere in the test arena. We transferred each squirrel from its home cage to a Tomahawk live trap (Tomahawk Live Traps, WI, U.S.A.) and released it into the test arena from the trap through a door in the arena wall. Each test lasted 10 min, which pilot work in *U. beldingi* and studies of boldness and antipredator behaviour in other species indicates is a sufficient amount of time (e.g. Bell & Stamps, 2004). The novelty of the arena may have affected squirrels' behaviours, but during the tests squirrels moved normally around the arena, including throughout the artificial burrow system. We measured the proportion of time they spent inside the refuge as an easily scored and clearly interpretable variable of antipredator behaviour, as more time in the refuge indicates stronger antipredator behaviour (Briffa et al., 2008; Rands, Cowlshaw, Pettifor, Rowcliffe, & Johnstone, 2003; Sih, Kats, & Maurer, 2003). To minimize observer effects we videotaped tests and scored them later blind to squirrel identity and treatment.

### Environmental Manipulations

Olfaction is a primary sensory modality of *U. beldingi* (Mateo, 2009), so we used odours to manipulate foraging and mating environments. We used odours from oestrous females to simulate a mating environment and odours of a favourite food to simulate a foraging environment. Although mating and foraging contexts are not strictly different levels of a single environmental variable, here we treated them as such. The natural history of *U. beldingi* supports

this interpretation. Males vary in seasonal activity by investing more time in mating activities early in the season followed by a greater investment in foraging activities later in the season (Sherman & Morton, 1984). We obtained oestrous female odours by trapping females in the wild on their day of oestrus, which we confirmed by direct observation of that female mating on that day, or observation of a sperm plug. Our primary trapping and sampling session occurred early in the afternoon, during which time we sampled odours of females suspected to be on their day of oestrus. Mating primarily occurs later in the afternoon until dusk, but we cannot exclude the possibility that a female mated early in the day prior to odour sampling, which means that male odours could potentially be mixed with the female oestrous odours. However, although this is a potential confound, our behavioural observations indicate it is unlikely to impact our results because female Belding's ground squirrels mate multiply and receive considerable attention from males even after numerous matings. To sample oestrous odours, we wiped the genital area of females with sterile cotton swabs. Males nasally inspect females' genital areas during oestrus (Mateo, n.d.). Likewise, in a closely related species of ground squirrel (Columbian ground squirrels, *Urocitellus columbianus*), males discriminate between odours from oestrous females and odours from anoestrous females (Harris & Murie, 1984). Both of these findings support our interpretation that these odours created a mating environment rather than a general social environment. After obtaining odours from an oestrous female on multiple swabs, we froze them, where they were kept for 1–3 weeks before use. Freezing effectively maintains odours for prolonged periods (Lenochova, Roberts, & Havlicek, 2009; Mateo, 2006; Scordato, Dubay, & Drea, 2007). For a behavioural test, the swabs were brought to room temperature and then multiple swabs were wiped on a 3 cm<sup>3</sup> polyethylene cube that had been previously washed in soapy water and sanitized using isopropyl alcohol (Mateo, 2009). We placed the odour cube in a PVC pipe covered with a wire mesh that allowed dissemination of odours but did not allow squirrels to contact the odour source, and we put the apparatus at the same location in the arena for every test. For the foraging environment, we used odours of peanut butter. Although this is not a natural food source, squirrels reliably and repeatedly eat peanut butter and investigate its odour. Both of the odour treatments induced robust behavioural responses (e.g. nasal investigations, object manipulation) indicating the odours were effective stimuli.

To concurrently manipulate a second environmental variable, we simulated an aerial predation event to mimic a high level of predation risk. After the first 5 min of the 10 min test, when the squirrel was outside the burrow, we threw a Frisbee over the test arena from a hidden position to simulate an attacking raptor. This type of aerial predator stimulus has been successfully used in previous studies of *U. beldingi* (Holekamp, 1986; Mateo, 2008; Mateo & Holmes, 1999). A closely related species (Richardson's ground squirrels, *Urocitellus richardsonii*), reacts to Frisbee predator simulations similarly to real aerial predation attempts (Davis, 1984). In response to the Frisbee in our test apparatus, squirrels fled to the burrow as *U. beldingi* do during real predation attempts, indicating that the Frisbee acted as a realistic predator stimulus. Therefore, we classified the approximate 5 min period after the predator stimulus as high predation risk due to the immediate threat of a known 'predator'. We classified the approximate 5 min period before the predator stimulus as low predation risk, because spending time outside the refuge still carries the threat of potential predators. Because the predator stimulus could only be presented when the squirrel was outside the burrow and not always at the exact 5 min mark, we used the proportion of time inside the refuge rather than just absolute time as our measure of antipredator behaviour.

### Experimental Design

We obtained four measurements of refuge use for each squirrel, one in the mating context and high predation risk, one in the mating context and low predation risk, one in the foraging context and the high predation risk, and one in the foraging context and low predation risk. Treating high/low predation risk as two levels of one variable and mating/foraging as two levels of another variable makes our experimental design a 2 × 2 factorial with repeated measures. We chose this design to mimic natural situations that male *U. beldingi* encounter and to quantify multidimensional plasticity and personality across a tractable set of environmental manipulations. We conceptualized the two levels of each variable as two points along an environmental gradient, such that the mating and foraging treatments, and high and low predation risk treatments, were treated the way one would treat temperature manipulations of 26 °C and 29 °C (e.g. Biro et al., 2010). Additional trials as controls (e.g. no scent, scents of nonoestrous females, Frisbee paired with a nonpredator bird call) would have provided added clarity and support regarding the impact of the treatments. However, our design adheres to the standard set in the behavioural syndromes and personality literature of comparing between levels of contexts (e.g. voracity of *Dolomedes triton* in a mating and foraging context; Johnson & Sih, 2005) and provides a clear quantification of the multidimensional plasticity and personality of squirrels' refuge use.

To obtain the four measurements of refuge use for each squirrel, we performed the test of refuge use described above on two separate test occasions. Each separate test occasion measured refuge use twice, once under high predation risk and once under low predation risk. On one test occasion, squirrels were presented with the mating odour treatment, and on the other occasion they were presented with the foraging odour treatment. In 2009, we tested 14 male squirrels. However, the order of foraging and mating manipulations was not counterbalanced. In 2010, we tested an additional 13 males, this time with a balanced order. When order was properly balanced in 2010, squirrels showed no order effects across test repetitions in both the high predation risk environment (paired *t* test:  $t_{12} = -0.82$ ,  $P = 0.43$ ) and low predation risk environment ( $t_{12} = 0.99$ ,  $P = 0.34$ ). Because this difference was not significant, we combined data from both years for analysis. In our data collection set-up, low predation risk always preceded high predation risk, because the time period following the aerial predator stimulus was defined as high predation risk. So while we were able to balance the order of the odour treatments, there was an order confound for the predation risk treatment. In a similar test apparatus, many squirrels became habituated and unresponsive to four repeated test occasions in a short period. This prevented an experimental design that balanced the order of all experimental combinations across separate test occasions. We acknowledge that this design shortcoming hampers interpretation of our results. To clarify our interpretations of results in light of this potential effect, we measured within-test order effects in a separate sample of 11 male squirrels. We compared refuge use in the first 5 min of the test versus the last 5 min, but with no predator stimulus. If squirrels spend more time in the burrow during the last 5 min due to an order effect, then the order effect will be confounded with our prediction of refuge use with respect to predation risk, preventing interpretation of the treatment effect. However, an order effect in the opposite direction, or no order effect, will allow interpretation of greater burrow use in the high predation risk treatment as due to the effect of the predator stimulus. We found significant differences in refuge use, but the effect was in the opposite direction of our prediction for antipredator behaviour in high and low risk manipulations (paired *t* test:  $t_{10} = -6.39$ ,  $P < 0.001$ ). Because our

predicted treatment effect is directional (greater refuge use under high predation risk), the order effect is not a confound, but it does prevent clear interpretation of effect size.

### Statistical Analysis

We used linear mixed-effects models to assess behavioural plasticity and animal personality of antipredator behaviour. We conducted our statistical analysis in R 2.15.2 using the `lmer` command in the `lme4` package. We arcsine transformed proportion of time in the refuge to improve how the residuals of the models fit normality and homogeneity of variance assumptions. We also standardized those values to clarify interpretation of coefficients (Schielzeth, 2010). Residual plots confirmed that the models using arcsine-transformed data did not violate model assumptions. Warton and Hui (2011) recently advised against arcsine transformation for proportional variables such as ours, recommending a binomial model using counts of successes and failures to comprise the proportion. However, when we used binomial models, residuals did not meet homogeneity of variance assumptions and were overdispersed, whereas the normal model using arcsine-transformed data had well-behaved residuals, so we report those here. Mixed-effects models allow assessment of plasticity through evaluation of the fixed effects terms, in this case the environmental manipulations, and they allow the assessment of personality through evaluation of a random term representing individual identity (Dingemans et al., 2010). Because each of the environmental variables is a two-level categorical variable, plasticity is the difference between values at those two levels rather than a true slope (Ensminger & Westneat, 2012). Therefore, as advocated by Ensminger and Westneat (2012), we coded mating and foraging treatments as  $-0.5$  and  $+0.5$ , and coded low predation risk and high predation risk treatments as  $-0.5$  and  $+0.5$ . This produces results that can be interpreted as slopes with the intercept centred at zero.

For the sake of comparing multidimensional plasticity to traditional unidimensional approaches, we ran multiple sets of models. We first ran mixed models evaluating personality and plasticity across each of the environmental manipulations separately, coding the particular environmental manipulation as a categorical fixed effect with two levels. Then we ran a full model including both environmental variables and their interaction as fixed effects. In each model, we included squirrel identity as a random intercept term and environmental manipulations as random slope terms (Schielzeth & Forstmeier, 2009). To test for significance of the random effects terms, we performed a likelihood ratio test between a model with the particular random effect and a simpler model without it, while maintaining the fixed effects structure (Crawley, 2007). We used the package `RLRsim` using the `exactLRT` function to calculate accurate  $P$  values when comparing models with a single random effect to models with no random effect (Crainiceanu & Ruppert, 2004). We obtained test statistics and  $P$  values for fixed effects after standardizing the response variable (Schielzeth & Forstmeier, 2009). We calculated degrees of freedom using traditional ANOVA methods on our sample of 108 observations on 27 individuals, which was appropriate since we had equal sample sizes across treatments.

We also tested whether male *U. beldingi* show consistent individual differences in plasticity across a secondary environmental variable. We wanted to explicitly test whether the individuals with greater plasticity of refuge use across predation risk treatments in the mating environment also had greater plasticity across predation risk treatments in the foraging environment. In other words, we tested for consistent individual differences in plasticity across environments. To test this, and the converse that individuals that

showed greater plasticity across mating and foraging treatments in the low risk environment also showed greater plasticity when in high risk, we calculated difference scores. A difference score is a simple way to quantify plasticity across two discrete levels of an environmental variable (Pigliucci, 2001). For example, we subtracted refuge use in the high risk manipulation from refuge use in the low risk manipulation. We calculated one difference score in the foraging odour treatment and another difference score in the mating odour treatment. Then we calculated the Spearman correlation between these two variables, as the difference scores were not normally distributed. To determine whether individual differences in plasticity to mating and foraging contexts were consistent across high and low predation risk, we subtracted refuge use in the mating environment from refuge use in the foraging environment for both high and low predation risk treatments and then calculated the Spearman correlations between the two variables. Our approach is similar to Morand-Ferron, Varennes, and Giraldeau (2011), except we did not use absolute value scores for plasticity because in our experimental set-up, the direction of plasticity is biologically relevant.

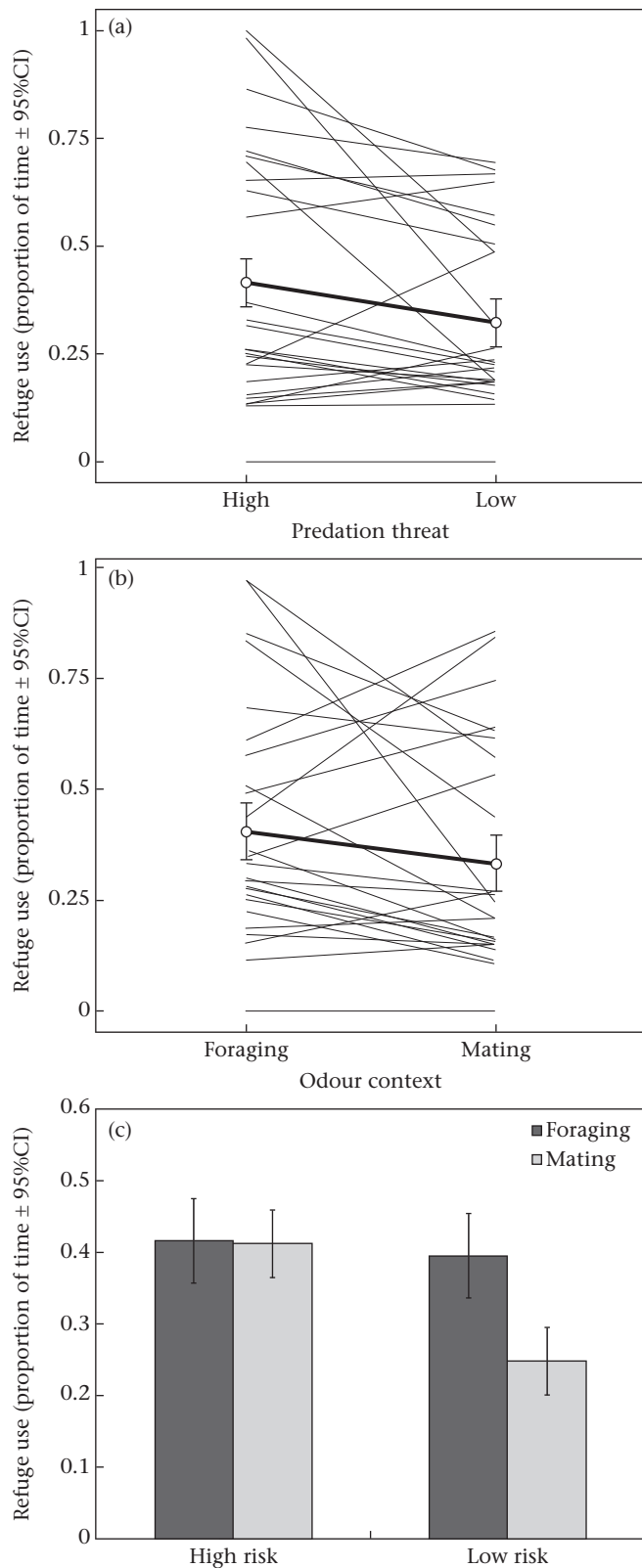
## RESULTS

### Plasticity of Antipredator Behaviour

On average, male *U. beldingi* spent about one-third of the test period in the refuge (mean  $\pm$  SD proportion of time in refuge =  $0.34 \pm 0.29$ ). Individuals changed their refuge use between environmental manipulations, with squirrels showing more refuge use in the high predation risk treatment than in the low risk treatment (Fig. 1a), and more refuge use in the foraging context than in the mating context (Fig. 1b). However, there were differences between unidimensional and multidimensional plasticity. When only main effects were included in the unidimensional models, squirrels significantly increased their refuge use from low to high predation risk (estimate  $\pm$  SE =  $0.292 \pm 0.119$ ,  $F_{1,26} = 5.42$ ,  $P = 0.03$ ; Fig. 1a) and they significantly decreased their refuge use from foraging to mating treatments (estimate  $\pm$  SE =  $-0.290 \pm 0.118$ ,  $F_{1,26} = 4.75$ ,  $P = 0.04$ ; Fig. 1b). In the multidimensional model the interaction term between the main effects was statistically significant (estimate  $\pm$  SE =  $-0.539 \pm 0.136$ ,  $F_{3,24} = 15.01$ ,  $P < 0.0001$ ; Fig. 1c). Squirrels spent the least amount of time in the refuge in the mating/low predation risk combination, differentiating between the odour treatments at low predation risk, but not at high predation risk (Fig. 1c).

### Personality of Antipredator Behaviour

Squirrels' refuge use was repeatable across the combinations of manipulated environmental variables. The random intercept term representing the variance attributed to animal personality was significant in all three sets of models (LRT 1a–c versus 2a–c; Table 1). The random slope terms representing individual variance in plasticity were not significant in models that tested the slope of a factor in the absence of another factor (LRT 2a versus 3a, 2b versus 3b, 2c versus 3c, and 2c versus 4c; Table 1). However, the random slope terms were significant for the model including both factors (LRT 3c versus 5c and LRT 4c versus 5c; Table 1). This difference shows that when a second variable is affecting individual plasticity, inclusion of that second random slope can increase power to detect significance of the first random slope. Last, the interaction of the two random slope components was not significant (LRT 5c versus 6c; Table 1). In other words, squirrels had significant individual differences in their responsiveness to each environmental variable, but not significant individual differences in their responsiveness to



**Figure 1.** Refuge use of male *U. beldingi* across multiple environmental variables: (a) unidimensional plasticity across high and low predation risk, (b) unidimensional plasticity across mating and foraging environments and (c) multidimensional plasticity evaluating combinations of environments. Confidence intervals ( $\pm 95\%$ ) were calculated using a correction for the within-subject design (Morey, 2008). In (a) and (b), the grey lines represent individuals' plastic responses across the environmental variable, while the black line represents the population mean plasticity. See text for statistical details.

particular combinations of those environmental variables. These results regarding random slope terms relate to consistent individual differences in plasticity that we address below.

#### *Consistent Individual Differences in Plasticity of Antipredator Behaviour across Secondary Environments*

Male *U. beldingi* showed consistent individual differences in plasticity when plasticity was measured in different environments. When considering the amount of plasticity shown across high and low predation risk, plasticity was significantly correlated between the foraging and mating environments ( $r_s = 0.427$ ,  $N = 27$ ,  $P = 0.03$ ; Fig. 2a). Likewise, when considering the amount of plasticity shown across mating and foraging environments, plasticity was significantly correlated between the predation risk manipulations ( $r_s = 0.597$ ,  $N = 27$ ,  $P = 0.001$ ; Fig. 2b). Because the difference scores factored out any among-individual variation due to the second variable, these results correspond to the tests of random slopes where the second variable is included to account for its variance (LRTs 3c versus 5c and 4c versus 5c; Table 1).

## DISCUSSION

In nature, animals encounter variation in a range of environmental variables. Mating, foraging, high predation and low predation risk environments present different costs and benefits to Belding's ground squirrels. When squirrels experienced different combinations of those variables, they showed significant multidimensional plasticity in refuge use, but also maintained consistent individual differences. The plastic response of squirrels' refuge use to an environmental variable was also repeatable when measured at different levels of a second environmental variable. To date, researchers have described behavioural variation across change in a number of environmental variables in many species (reviewed in: Betini & Norris, 2012; Briffa, Bridger, & Biro, 2013; Carter, Goldizen, & Heinsohn, 2012; Dingemans et al., 2010; Rodríguez-Prieto, Martín, & Fernández-Juricic, 2011). We know less about behavioural variation in response to change in combinations of environmental variables (but see Stahlschmidt & Adamo, 2013; Stahlschmidt & DeNardo, 2010; Westneat et al., 2011). Our results are consistent with previous reports of personality and plasticity, but indicate complex patterns of variation when behaviour is repeatedly expressed across combinations of multiple environmental variables.

Belding's ground squirrels' antipredator behaviour varied in response to the environmental manipulations. As expected, male *U. beldingi* showed more refuge use in a situation indicating high predation risk than in one indicating low predation risk (Fig. 1a). We predicted that male *U. beldingi* would use the refuge less in the mating environment than in the foraging environment due to the greater evolutionary payoff of a potential mate versus a potential meal. This prediction assumes that individuals are sufficiently fed and in good condition, which was the case with our laboratory feeding regime but may not be the case in nature. We also add caution to our functional interpretation of the effect of this factor because we compare refuge use between these two levels rather than comparing how each differs from a no-scent control. Still, the model comparing only the foraging environment to the mating environment supported our prediction (Fig. 1b). However, these two results obtained from traditional unidimensional approaches to phenotypic plasticity do not adequately describe the full range of variability attributed to the environmental manipulations. The multidimensional model showed a significant interaction effect of the environmental manipulations on antipredator behaviour (Fig. 1c). In other words, the effect of one environmental

**Table 1**  
Model comparison testing the significance of random effect terms in unidimensional models testing plasticity across foraging and mating environments (models 1a–3a), unidimensional models testing plasticity across high and low predation risk environments (models 1b–3b) and models evaluating multidimensional plasticity across combinations of environmental variables (models 1c–6c)

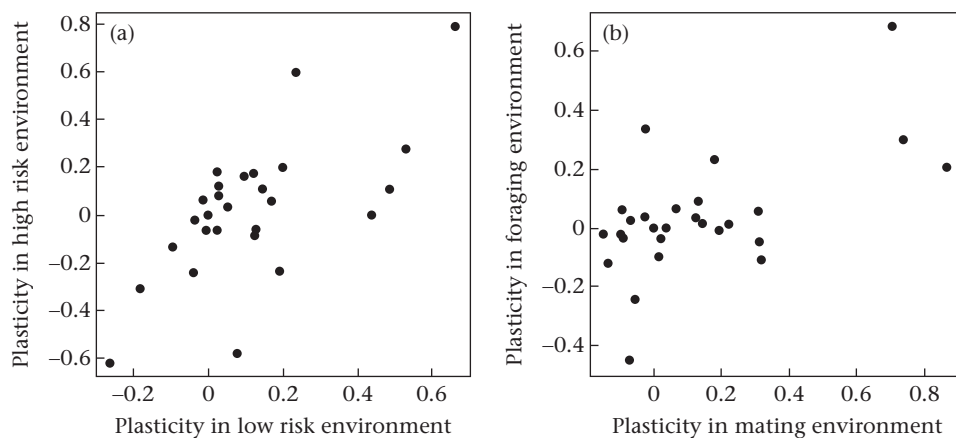
	Model	Fixed effects	Random intercept	Random slopes	Likelihood	Test	LRT	df	P
Unidimensional plasticity	1a	Odour	—	—	−153.8				
	2a	Odour	ID	—	−127.4	1a vs 2a	51.42	1	<b>&lt;0.001</b>
	3a	Odour	ID	Odour	−127.0	2a vs 3a	0.98	2	0.614
Unidimensional plasticity	1b	Pred	—	—	−153.8				
	2b	Pred	ID	—	−127.4	1b vs 2b	50.70	1	<b>&lt;0.001</b>
	3b	Pred	ID	Pred	−125.4	2b vs 3b	4.08	2	0.130
Multidimensional plasticity	1c	Pred*Odour	—	—	−152.5				
	2c	Pred*Odour	ID	—	−121.3	1c vs 2c	52.82	1	<b>&lt;0.001</b>
	3c	Pred*Odour	ID	Odour	−119.7	2c vs 3c	3.30	2	0.19
	4c	Pred*Odour	ID	Pred	−118.9	2c vs 4c	4.95	2	0.08
	5c	Pred*Odour	ID	Pred+Odour	−113.1	3c vs 5c	13.29	3	<b>0.004</b>
	6c	Pred*Odour	ID	Pred*Odour	−109.3	4c vs 5c	11.65	3	<b>0.009</b>
						5c vs 6c	7.60	4	0.13

Odour: categorical variable representing mating and foraging manipulations; Pred: categorical variable representing high and low predation risk manipulations; ID: squirrel identity; LRT:  $\chi^2$  value for the log likelihood ratio test. Significant *P* values (<0.05) are shown in bold.

manipulation depended on the level of the other. Male *U. beldingi* spent the least time inside the refuge in the mating/low predation combination. Squirrels showed greater differences in the proportion of time spent in the refuge between the mating and foraging environments in the low predation risk environment. They spent more time in the refuge in the high risk environment, but did not differentiate between foraging and mating environments when under high risk. The interaction effect may occur due to a balance between current and future reproductive efforts. Males have multiple mating opportunities within and across seasons, and so the lack of differences in the proportion of time using the refuge between mating and foraging treatments in the high predation risk environment may reflect a conservative tactic to ensure survival to future reproductive opportunities. Both environmental variables are relevant to explaining variation in antipredator behaviour of male *U. beldingi*. However, the interaction term overlooked by unidimensional models is also important, and a potentially adaptive facet of behavioural variation in this species. Multidimensional plasticity may be adaptive in other species, such as the interaction between brood size and nestling age on parental behaviour in house sparrows (Westneat et al., 2011). Also, in Children's pythons, *Antaresia childreni*, temperature and humidity have an interactive effect on parental behaviour (Stahlschmidt & DeNardo, 2010).

Together, these results demonstrate the benefit of addressing combinations of environmental variables and their effect on behaviour.

Despite the multidimensional plasticity shown by male *U. beldingi*, we still found consistent individual differences in squirrels' refuge use across all combinations of environmental manipulations. The consistent individual differences in refuge use may be due to genetic polymorphisms arising from selective pressures, but an additional potential explanation is that personality reflects differences due to environments that the individuals experienced prior to our manipulations. Development influences animal personality (Duckworth, 2010; Sih, 2011; Sih et al., 2004; Sinn, Gosling, & Moltschanowskyj, 2008; Stamps & Groothuis, 2010), and our results point towards more complex effects of plasticity on personality. Stamps and Groothuis (2010) noted that the term behavioural plasticity is applied indiscriminately to when behaviour changes as a function of current environments or previously experienced environments. A plastic response can persist and the behavioural effect remains in the absence of, or change in, the environmental variable. Conversely, a plastic response may be ephemeral, and the effect on behaviour changes once the current environment changes. If an environment's effect on behaviour is lasting, differences in the time of onset (Butler, Toomey, McGraw, &



**Figure 2.** Consistent differences in individual plasticity across different environmental contexts. (a) The difference score (i.e. plasticity) between refuge use in the mating environment and the foraging environment was calculated in both high and low predation risk environments and the plot shows their correlation. (b) The difference score between refuge use in the high and low predation risk environment was calculated in both foraging and mating environments and the plot shows their correlation. Both correlations were significant ( $P < 0.05$ ).

Rowe, 2012; van de Pol & Cockburn, 2011) and differences in the duration of response to that environment can manifest themselves as among-individual variance (i.e. personality). For example, one squirrel's response to an oestrous female the previous day may be more persistent than that of another squirrel, which would impact both personality and plasticity of refuge use due to multidimensional plasticity. Westneat et al. (2009, 2011) found multidimensional plasticity and consistent individual differences in clutch size and parental care in a population of free-ranging house sparrows, including plasticity along temporal variables. These findings and our results point towards the need to address how environmental variables change over time, in addition to addressing interactive effects of multiple environmental variables on behaviour.

We also found consistent individual differences in plasticity of antipredator behaviour (Fig. 2). Squirrels' change in refuge use between mating and foraging manipulations was consistent across high and low predation risk (Fig. 2a), and their change in refuge use between high and low predation risk was consistent across mating and foraging environments (Fig. 2b). We found no significant individual differences in the slope interaction term, although that may be due to low statistical power (Table 1). Morand-Ferron et al. (2011) analysed the plasticity of nutmeg mannikins', *Lonchura punctulata*, foraging behaviour in patch choice and producer–scrounger games and did not find individual plasticity to be correlated across the two games, but did find consistency over time. Here we supplement those results to include consistent individual differences in plasticity across other environmental variables. Although asking whether plasticity is repeatable may seem counterintuitive, this question is relevant to the evolution of labile traits. Multiple studies have uncovered significant variation in behavioural plasticity among individuals, in some cases showing repeatability of plasticity over time or significant variation in behavioural plasticity to more than one environmental variable (Biro et al., 2010; Carter et al., 2012; Mathot et al., 2011; Westneat et al., 2011). In line with those results, here we show repeatability of behavioural plasticity over time to two environmental variables. However, by measuring each individual in all possible environmental combinations and assessing the correlation between difference scores, our results are unique in that they explicitly show that individuals' behavioural plasticity to a given environmental variable is repeatable across multiple levels of other environments. As a given environmental variable changes, the level of other environmental variables can impact response to that change (Fig. 1c), yet individuals' responses remain consistently different irrespective of the level of the other environmental variable (Fig. 2a, b). This repeatability of plasticity will impact how selection operates on that variation. For male *U. beldingi*, the plasticity of antipredator behaviour was a consistent trait with the potential to be a target of selection.

Animals respond to their environments behaviourally, and understanding the complexity of those responses will shed light on the evolution of behaviour. Here we provide data that show multidimensional plasticity of antipredator behaviour in male Belding's ground squirrels in response to manipulated combinations of mating and foraging and high and low predation risk environments. Consistent individual differences in behaviour, or animal personality, persisted across manipulations of combinations of environmental variables. Furthermore, squirrels showed consistent individual differences in plasticity when plasticity in response to one environmental variable was measured at different levels of a second environmental variable. Together, these results clarify patterns of behavioural variation in response to complex, realistic environmental variation animals encounter in nature. A number of traits other than behavioural traits respond to such environmental variation, and so these data should benefit a variety

of fields in addition to animal personality, such as evolutionary physiology (Zera, Harshmann, & Williams, 2007) and life-history theory (Nussey, Wilson, & Brommer, 2007).

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