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Play behavior and the development of boldness and caution in juvenile Belding's ground squirrels (*Urocitellus beldingi*)

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ABSTRACT

The ubiquity of play among juvenile mammals suggests it provides adaptive benefits, potentially through influences on the development of temperament in young animals. Juvenile Belding's ground squirrels (*Urocitellus beldingi*) must balance competing demands for boldness and caution imposed by the fundamental trade-off between their short active season and their vulnerability to predation. In this study, I evaluated whether play helps to facilitate the development of an appropriate balance between boldness and caution in juvenile *U. beldingi.* I observed the play behavior of juvenile *U. beldingi* and conducted flight-initiation distance tests to measure boldness-caution at the beginning and toward the end of the developmental period during which play primarily occurs. I recorded the distances at which juveniles responded to intruders and the vigilance displayed by juveniles during tests as measurements of boldness-caution. The age of mothers was a significant predictor of the outcome of initial tests. Juveniles with yearling mothers responded to intruders at greater distances than did juveniles with older mothers. Juveniles exhibited increased caution in response to intruders over the play period. Age of mothers was a significant predictor of increases in vigilance displayed by juveniles over the play period, with juveniles with younger mothers having the largest increases. These results suggest maternal influences on the development of boldness-caution, with young of less experienced mothers developing greater caution. Rates of social play were significant predictors of increases in distances to notice and flee from an intruder over the play period, with juveniles who engaged in social play at the highest rates having the greatest increases. Other studies have indicated an association in some situations between social play and increased boldness rather than greater caution. Together with these studies, my results suggest that possible influences of play behavior on boldness-caution may vary with the context in which an animal finds itself.

INTRODUCTION

Play Behavior

Traditional definitions describe play behavior as actions that have no obvious immediate function and may seem frivolous in the context in which they are expressed (Fagen 1981; Palagi 2018). However, Burghardt (2005) helped to provide clarity to that definition by describing five common elements of play that can be used as criteria for more specifically classifying behaviors as play: (1) play behaviors are voluntary and spontaneous; (2) within a species, play behaviors are characteristic of the species and are expressed repeatedly but not invariantly by individuals; (3) play is not completely functional in the context in which it occurs, but in some cases may resemble functional behaviors; (4) when play does resemble functional behavior, it is a modified version of the behavior and does not generate the same outcome or objective as the behavior it resembles; and (5) play behaviors are expressed by healthy, unstressed individuals and do not include stereotypically aberrant actions that may result from stress. The seemingly "frivolous" nature of play raises questions about why animals engage in play behavior. These questions can address the evolutionary origins of play behavior across taxa or the functional benefits that individuals gain from engaging in play (Pellis et al. 2015). In this work I evaluated possible adaptive benefits of play behavior, using Belding's ground squirrels (*Urocitellus beldingi*) as a model system. In particular, I evaluated the hypothesis that play behavior in juvenile *U. beldingi* influences the expression of adaptive behavioral responses in terms of boldness and caution in a variety of contexts.

Play behavior can be social, involving interactions between individuals, or non-social, involving locomotor actions, such as running or climbing, or interactions with objects in the surrounding environment (Fagen 1981; Burghardt 2005). Play behavior, though generally rare, has arisen multiple times across a range of animal taxa.

Play behavior is notably prevalent among mammals: young of nearly all mammalian species engage in play behavior at some point during their development (Burghardt 2005; Pellis et al. 2014, 2015). Despite the ubiquity of play among mammals, a number of costs are thought to be associated with this behavior. While the overall costs of play behavior are largely unknown (Graham and Burghardt 2010), engaging in play does require an expense of time and energy. Additionally, during play, animals may be distracted and may behave in a more conspicuous manner, potentially increasing their vulnerability to predation (Whishaw et al. 2001). In mammals, maternal care such as provisioning offspring via milk and protecting offspring from threats can help to offset some of the costs associated with play, which may contribute to the prevalence of play among mammalian species (Burghardt 2005; Pellis et al. 2014). However, given what is known about natural selection, the ubiquity of play among juvenile mammals suggests that play provides benefits to young animals that outweigh the potential costs. Thus, it is pertinent to discuss the adaptive benefits of play.

Functional benefits of engaging in play behavior can vary among species and between sexes within a species (Olioff and Stewart 1978; Meder 1990; Pederson et al. 1990; Pellis et al. 1996, 2014; Bekoff and Byers 1998; Nunes et al. 1999; Mastripieri and Ross 2004; Burghardt 2005; Paukner and Suomi 2008; Auger and Olesen 2009). The adaptive benefits of play may be short-term and limited primarily to the juvenile period, or longer-term, extending into adulthood (Held and Spinka 2011; Blumstein et al. 2013; Nunes 2014; Ahloy Dallaire and Mason 2017). The majority of studies on play behavior have focused on understanding its short-term benefits. Various studies of mammals have suggested that play behavior promotes motor, social, and cognitive development in young animals (Bekoff and Byers 1998; Pellis et al. 2010; Kuczaj and Eskelinen 2014). For example, play behavior improves motor coordination in juvenile *U. beldingi* (Nunes et al. 2004a, 2004b). Play is also associated with improved motor skills, social and communicative competence, and problem-solving abilities in various species of dolphins (Kuczaj and Eskelinen 2014). Some recent studies have also identified long-term benefits to play. In brown bears (*Ursus arctos*, Fagen and Fagen 2004, 2009) and horses (*Equus caballus*, Cameron et al. 2008), juvenile play behavior is correlated with increased long-term survival. In yellow-bellied marmots (*Marmota flaviventris*), play promotes the establishment of social hierarchies that are stable across the lifespan of individuals (Blumstein et al. 2013). In American minks (*Neovison vison*), juvenile play among young males is associated with enhanced sexual proficiency in adulthood (Ahloy Dallaire and Mason 2017). Moreover, in *U. beldingi*, juvenile play among young females is associated with increased likelihood of establishing maternal territories and successfully weaning litters as yearlings (Nunes 2014).

Spinka et al. (2001) suggested that an important function of play behavior may be to train young animals to deal with unexpected or unfamiliar situations by influencing the development of kinematic and emotional flexibility. Work with laboratory rats (*Rattus norvegicus*) has provided some support for this hypothesis, by identifying associations between play behavior and increased behavioral flexibility (Pellis et al. 2014; Himmler et al. 2016). Further support has come from a recent study by Marks et al. (2017), which evaluated juvenile play behavior in a free-living population of Belding's ground squirrels (*Urocitellus beldingi*).

Play and Temperament

Spinka et al. (2001) further proposed that play might help prepare young animals for the unexpected via its effects on the development of temperament. Temperament is defined as the behavioral tendencies of an individual that are repeatable over time or across contexts (Reale et al. 2007). Possible influences of play on the development of temperament in young animals may in turn guide the way that young animals respond in unfamiliar situations. The type and degree of emotional response an animal exhibits may have an impact on the outcome of an interaction or novel situation; for example, emotional overreaction may lead to an escalation of conflict during an agnostic encounter (Spinka et al. 2001).

Elements of temperament ("temperament traits") can include individual tendencies toward docility, boldness, aggression, or exploration (Reale et al. 2007; Conrad et al. 2011; Rasmussen and Belk 2016). Temperament traits have been shown to affect a wide variety of ecologically important variables such as reproductive success, survival, and physiological responses to stress (e.g. Carlstead et al. 1999; Carere et al. 2001; Spinka et al. 2001; Dingemanse et al. 2004; Both et al. 2005; Cavigelli et al. 2006; Boon et al. 2007; Boon et al. 2008; Hulbert et al. 2011; Plush et al. 2011; Baugh et al. 2013; Vetter et al. 2016; Colchester and Harrison 2016).

A number of factors may work together to determine an individual's temperament, and different temperaments may be adaptive in different contexts. For example, boldness and caution, which refer to an individual's propensity to take risks or engage in risk-aversive behaviors, respectively, when faced with novel or threatening situations, may be favored in different contexts. *Cautious* individuals take fewer risks than *bold* individuals; therefore, these temperaments may be differentially favored in predators and prey (Reale et al. 2007; Conrad et al. 2011; Rasmussen and Belk 2016; Chang et al. 2017; Petelle et al. 2017). In predatory species, bolder individuals may have an advantage over more cautious conspecifics due to improved foraging success (e.g. Sundström et al. 2004), while in prey species, bolder individuals may be at a disadvantage due to reduced survivorship (e.g. Biro et al. 2004).

Different temperaments may also be favored under different environmental conditions. For instance, docility – an individual's lack of responsiveness in a situation, occurring along a continuum from passive to active responses (Reale et al 2007; Hurst-Hopf et al. in prep) – may be differentially expressed based on environmental stability. In unstable, unpredictable environments, natural selection may favor individuals with reactive temperaments who tend to be more passive, taking time to gather information about their situation, then choosing a response based on the specific circumstances of their situation. By contrast, in stable environments with predictable parameters, natural selection may favor individuals with proactive temperaments who tend to take a more preventative approach, quickly responding to each situation to prevent potential problems before they arise.

Maternal influences can have an impact on the development of temperament in young animals. For example, in yellow-bellied marmots, glucocorticoids transferred to offspring through mother's milk decreases docility in offspring (Petelle et al. 2017). Moreover, concentrations of circulating glucocorticoids can vary with environmental factors such as population density (Dantzer et al. 2013). Maternal effects on development may enhance offspring fitness by adjusting offspring phenotypes to be compatible with the prevailing environmental conditions (Dantzer et al 2013; Petelle et al. 2017).

Spinka et al. (2001) suggested that play behavior may also have a role in fine-tuning the development of temperament in young animals. Preliminary support for this hypothesis was provided by a recent study of the relationship between social play behavior and the development of docility in juvenile *U. beldingi* (Hurst-Hopf et al. in prep). Juvenile squirrels who engaged in social play at higher rates exhibited greater decreases in docility across development, a finding that suggests social play may help to fine-tune the expression of docility, possibly allowing juveniles to display more appropriate responses in specific situations. Hurst-Hopf et al. (in prep) further observed that rates of social play among juvenile squirrels were reliable predictors of their docility as yearlings, suggesting that by fine-tuning the expression of docility in young animals, play may promote the expression of context-appropriate responses later in life.

Docility and boldness-caution have both been observed to change across the lifespan of individuals; however, evidence from studies on yellow-bellied marmots suggests that boldness-caution may be more flexible over time than docility (Petelle et al. 2013). If boldness-caution is more malleable than docility, influences of play on the development of temperament might be expected to produce more observable variations in boldness-caution than docility.

Belding's Ground Squirrels

The range of *U. beldingi* extends across high-elevation meadows in the western United States. Following a very short growing season during late spring and summer, their habitat is covered in snow for the remainder of the year. Consequently, *U. beldingi* have a prolonged period of hibernation, lasting approximately nine months (Jenkins and Eshelman 1984). The squirrels emerge from hibernation as the last of the winter snowpack melts at the onset of the growing season in late spring, and they are active for about three months. Mating begins soon after the squirrels emerge from hibernation, and gestation lasts 25 days. Since *U. beldingi* have such a short active period, females bear at most one litter each year (Jenkins and Eshelman 1984). Newborn squirrels remain underground in natal burrows during lactation and first appear above ground between 25-28 days of age, near the time of weaning (Holekamp et al. 1984; Nunes et al 1999). During their first two weeks above ground, juveniles undergo a well-defined period of development during which the majority of play behavior occurs in this species, with social play (> 97% of interactions) occurring mainly among littermates (Nunes et al. 1999). This well-defined *play period* sets up an ideal system for studying the interaction between play behavior and juvenile development.

Reproductive female *U. beldingi* defend maternal territories during gestation, lactation, and throughout the juvenile play period; they aggressively evict intruders from their territories.

As small, herbivorous mammals, *U. beldingi* are prey to a range of predators, with juveniles being especially vulnerable to predation (Sherman 1977; Jenkins and Eshelman 1984). Juveniles also face an additional threat of infanticide from adult and yearling conspecifics who attempt to invade their natal territories (Sherman 1981). While maternal care partially offsets juveniles' vulnerability to these threats, juvenile *U. beldingi* still suffer from high mortality rates (Sherman and Morton 1984).

Boldness-caution in U. beldingi

Juvenile *U. beldingi* are faced with two competing demands. On one hand, the squirrels have a short amount of time to grow and learn prior to the onset of hibernation, which may favor bolder temperaments that facilitate greater foraging and exploration. On the other hand, juvenile squirrels are highly vulnerable to predation, which may favor more cautious temperaments that reduce the likelihood of mortality from predation. Consequently, juvenile *U. beldingi* must find a way to balance this fundamental trade-off between the pressure to be bold to derive optimal benefits from their habitat during the growing season and the competing need to be cautious to reduce the risk of predation. Previous associations found between juvenile play behavior and the development of docility in *U. beldingi* (Hurst-Hopf et al. in prep) give rise to the idea that play may also be facilitating a balance between boldness and caution in *U. beldingi*.

The overall goal of this study was to explore the possibility that play in *U. beldingi* influences the development of caution in response to perceived threats, and boldness in response to novelty. Marks et al. (2017) observed that social play in juvenile *U. beldingi* was associated with the development of bold responses in novel situations; juveniles who engaged in social play at higher rates and had longer duration play bouts exhibited greater increases in exploration in an unfamiliar environment and greater decreases in time to escape from a novel testing arena across the play period. The results of Marks et al. (2017) suggest that juveniles' boldness in response to novelty may increase over the play period, supporting the idea that *U. beldingi* benefit from being bold in unfamiliar situations. Moreover, the results of Marks et al. (2017) suggest that play behavior may promote expression of bolder responses in unfamiliar situations. Following up on the work by Marks et al (2017), the present study aimed to assess whether social play in juvenile *U. beldingi* is also associated with the development of increased caution in response to perceived threats. Expanding on the research of Hurst-Hopf et al. (in prep), I evaluated correlations between natural variations in social play and the development of boldness-caution in juvenile *U. beldingi*.

MATERIALS AND METHODS

From May-July 2018 I studied a population of *U. beldingi* at Tioga Pass (37° 55' N, 119° 15' W) in Mono County, California, United States. I observed the maternal territories of reproductive females daily to determine the dates that their litters first emerged from the natal burrow. At their first emergence from the natal burrow, juvenile squirrels were captured using Tomahawk live-traps (Tomahawk Live-Trap Company, Hazelhurst, Wisconsin) baited with peanut butter. Captured juveniles were fitted with numbered ear tags (National Band and Tag Company, Newport, Kentucky) for permanent identification and given unique, distinguishing marks with hair dye to aid in identification during observations. Ear tags were painted different colors with nail polish to aid in identifying individuals during observations. Prior work with *U. beldingi* indicated that colored ear tags do not influence the play behavior of juveniles (Nunes et al. 2015). At their first capture, juveniles were weighed with spring balance scales (Avinet, Dryden, New York). Traps were checked every thirty minutes or less during trapping sessions, and juveniles were returned to their natal burrows after handling. Trapping and other methods in this study followed guidelines published by the American Society of Mammalogists (Sikes et al. 2016).

Behavioral Tests

I conducted behavioral tests on juvenile squirrels to gauge boldness-caution in response to an intruder at the beginning and toward the end of the play period; I used a modified flight-initiation distance test (Runyan and Blumstein 2004; Petelle et al. 2013), a previously established test which measures the distance at which an individual first flees from an approaching human (Ydenberg and Dill 1986; Blumstein 2003). Flight is an anti-predatory behavior, and flight-initiation distance is commonly used as an inverse measure of individual boldness – or a direct measure of caution (Cooper 2009; Petelle et al. 2013). I conducted tests at first emergence from the natal burrow for a total of 89 juveniles from 21 different litters and then 12-13 days later for 71 of those juveniles, toward the end of the play interval. Of the 89 juveniles initially tested, 18 disappeared before they could be re-tested. The 21 initial litters were all represented among the 71 juveniles who were re-tested. At the initiation of tests, the juveniles being tested (1) were approximately 20 meters away from the person conducting the test (henceforth the "intruder"), (2) had been feeding and/or resting (Table 1) continuously for at least five minutes, (3) were not within two meters of any other squirrels, and (4) were within 1.5 meters of a burrow entrance that could be used for escape. During tests, the intruder walked toward the target squirrel at a constant pace of 0.5 m/sec. Intruders were trained to walk at a consistent pace prior to conducting tests. Tests were considered completed when a squirrel fled from the intruder.

Each intruder logged notes during tests using a digital voice recorder, which also recorded time. Intruders made note of the points at which (1) each test began, (2) the squirrel first noticed the intruder (3) the squirrel expressed a specific vigilant behavior (Table 1; head up, sit up, or post), and (4) the squirrel fled from the intruder. A squirrel was considered to have noticed an intruder when it turned its head toward the intruder or displayed a vigilant behavior. Each intruder dropped flags during each test to mark their location when first noticed by the squirrel and their location when the squirrel fled. At the end of each test, the intruder marked the initial location of the squirrel with a flag. Distances between flags were later measured to determine how far the squirrel was from the intruder when it first noticed the intruder, and how far away it was when it fled.

I also observed the vigilance displayed by juveniles during intruder tests. Vigilance refers to anti-predatory behaviors that make an individual more aware of its surroundings and potential threats (Nunes et al. 1997, 2000) and was considered to be a direct measurement of caution. I gave non-vigilant behavior during a test a score of 1. I assigned scores for vigilant behaviors as follows, with higher intensity behaviors having higher scores: head up—2, sit up—3, post—4 (Table 1). I expressed the amount of time each squirrel displayed different behaviors as a proportion of the time that squirrel was aware of the intruder before fleeing. For each squirrel, I then produced an overall intensity score for vigilance by summing the products obtained by multiplying the proportion of time the squirrel displayed each behavior by that behavior's assigned score. I also calculated the total amount of time each squirrel expressed vigilant behavior as a proportion of the time it was aware of the intruder before fleeing.

Observation of Behavior

I observed the behavior of 71 juvenile *U. beldingi* for whom I could conduct both initial and follow-up intruder tests (see *Behavioral Tests*). I conducted behavioral observations 0700 and 1600 hours during the two week period following first emergence of juveniles from the natal burrow. I observed juveniles for a total of 387.3 ± 17.7 minutes on average, over 5.8 ± 0.1 different days. During observations, I recorded all occurrences of social play behavior and noted the duration of each play bout. I calculated rates of social play for each juvenile as the number of play interactions per hour of observation. Table 2 lists definitions of specific *U. beldingi* play behaviors based on descriptions by Marks et al. (2017).

Statistical Analysis

I evaluated possible correlations between natural variations in social play and changes in boldness-caution across the play period. If caution in response to perceived threats from intruders is an advantageous strategy in *U. beldingi*, then juveniles' caution in response to intruder tests should increase over the course of the play period; furthermore, if social play in *U. beldingi* influences the development of increased caution in response to perceived threats from intruders, then juveniles who engage in larger amounts of social play should exhibit greater increases in caution across the play period.

Continuous variables evaluated in the study included distances to notice and flee from an intruder and changes in these distances, time spent vigilant and vigilance intensity scores and changes in these variables, body mass, age of mothers, sex ratio within litters, rates of social play, durations of play bouts, and the number of play partners. Among these, durations of social play bouts and their residuals did not follow the normal distribution. However, following logarithmic transformation (transformed value = ln [original value]) of durations of play bouts, their values and residuals followed the normal distribution and met assumptions of statistical tests. I used Pearson's *r* to evaluate correlations between variables. I compared data between juvenile males and females using independent *t*-tests. I also used independent *t*-tests to compare initial values of distances to notice and flee from an intruder and vigilance scores between juveniles who survived and disappeared throughout the play period; I omitted a litter of seven juveniles from the analysis because they all disappeared at the same time, suggesting a single event occurred resulting in the disappearance of the entire litter rather than seven separate instances of disappearance of juveniles in the litter. I used paired *t*-tests to compare initial and re-test values of distances to notice and flee from an intruder and vigilance scores. The data used in *t*-tests met assumptions of homoscedasticity. Multiple linear regression with forward selection was used to determine whether body mass, age of mothers, or sex ratio within litters were reliable predictors of distances to notice or flee from an intruder or vigilance scores in initial behavioral tests. Forward selection begins with an empty model and adds in variables one by one. In each forward step, the statistical program adds in the single variable that produces the best model based on Akaike's Information Criteria. I also used multiple linear regression to determine whether body mass, age of mothers, rates and durations of social play bouts, and the number of play partners were reliable predictors of changes in distances to notice or flee from an intruder or changes in vigilance scores across the play interval. I further analyzed the variables identified in each multiple linear regression to be reliable predictors of the results of behavioral tests by including them as fixed effects in mixed linear models, with litter included as a random effect to account for any similarities among juveniles from the same litter. Analyses using mixed linear models met assumptions of homoscedasticity. Following analyses with mixed linear models, I conducted Tukey's HSD tests for pairwise comparisons. I used Systat 13.1 (Systat Software, Inc., Chicago, Illinois, USA) to perform all statistical tests. I considered relationships indicated by statistical tests to be significant when $p \leq 0.05$. For ease of interpretation, all the graphs included in this report present non-transformed values of variables.

RESULTS

Initial Responses to Intruder Tests

The distances at which juveniles noticed and fled from an intruder, the time spent vigilant, and the intensity of vigilance during behavioral tests at first emergence from the natal burrow did not differ between the sexes or between juveniles who survived and disappeared. Multiple linear regression was used to determine whether any of the following variables – individual body mass, sex ratio within litters, and the age of mothers – were significant predictors of distances to notice or flee from an intruder, time spent vigilant, or intensity of vigilance. None of the variables in this analysis were significantly correlated with any others ($-0.15 < r < 0.06$). Values of variables did not differ by sex, so data were combined for juvenile males and females.

Multiple regression indicated a significant relationship ($F_{1,87} = 6.58$, $p = 0.012$) between the initial distance to notice an intruder and the age of mothers ($t = 2.57$, $p = 0.012$) as well as a significant relationship ($F_{1,87} = 6.01$, $p = 0.016$) between the initial distance to flee from an intruder and the age of mothers ($t = 2.45$, $p = 0.016$). I further evaluated the relationship between the age of mothers and the distances at which juveniles noticed and fled from an intruder during initial tests, incorporating prior maternal experience into the analyses. Mothers were divided into three groups based on age and parity (Nunes 2014): primiparous yearlings, primiparous adults, and multiparous adults. Data were evaluated using mixed linear models with grouping of mothers as a fixed effect and litter as a random effect. The distances at which juveniles noticed an intruder during initial tests varied significantly with mother's age/parity grouping (Fig. 1A, $F_{2,68} = 3.42$, $p = 0.038$), as did the distance at which juveniles fled from an intruder during initial test (Fig. 1B, $F_{2,68} = 3.27$, $p = 0.044$). In particular, the distances at which juveniles noticed and fled from intruders during initial tests tended to decrease as the age and parity of their mothers increased (Fig. 1).

Multiple linear regression also indicated a significant overall relationship ($F_{1,87} = 9.00$, $p = 0.004$) between the time juveniles spent vigilant during initial tests and body mass ($t = 3.00$, $p = 0.004$) as well as a significant relationship ($F_{1,87} = 5.73$, $p = 0.019$) between the intensity of vigilance expressed during initial tests and body mass ($t = 2.39$, $p = 0.019$). I further evaluated the relationship between juvenile body mass and vigilance at first emergence from the natal burrow. The range of body masses observed among the 89 juveniles in this part of the study (50-104 grams) was divided into equal thirds to assess juveniles with the lowest, intermediate, and highest masses. Vigilance data was evaluated using a mixed linear model with body mass groupings of juveniles as a fixed effect and litter as a random effect. This analysis indicated that the intensity of vigilance expressed during initial tests among juveniles varied significantly with their body mass (Fig. 2, $F_{2,67} = 5.30$, $p = 0.007$). In particular, the intensity of vigilance at first emergence from the natal burrow tended to be greater among heavier juveniles (Fig. 2).

Changes in Responses to Intruder Tests Across the Play Interval

The distance to notice an intruder increased significantly among juveniles between initial behavioral tests, conducted at first emergence from the natal burrow, and re-tests near the end of the play period (Fig. 3A, $t_{70} = 8.10$, $p < 0.001$), as did the distance to flee from an intruder (Fig. 3B, $t_{70} = 6.85$, $p < 0.001$). The proportion of time spent vigilant during tests also increased significantly across the play period (Fig. 3C, $t_{70} = 3.96$, $p < 0.001$); however, the intensity of vigilance expressed during behavioral tests did not change significantly among individuals across the play interval.

Stepwise multiple linear regression was used to evaluate whether changes in the distance to notice or flee from an intruder or changes in the time spent vigilance during tests were associated with body mass at first emergence from the natal burrow, the age of mothers, or play-related variables (rates and durations of play bouts). Sex ratio within litters was not included in this analysis because 18 of the initial 89 juveniles in the study disappeared during the play interval, causing sex ratio not to be constant in some litters. None of the independent variables in these analyses were significantly correlated with any others ($-0.21 < r < 0.26$). Changes in distances to notice or flee from an intruder were expressed as proportions of initial distance. Changes in time spent vigilant were not expressed as a proportion of the initial time spent vigilant because some of the initial times were zero. There were no sex differences in any of the dependent or independent variables evaluated, so data were combined for juvenile males and females.

Among the independent variables evaluated, stepwise multiple regression indicated a significant overall relationship ($F_{1,69}$ = 11.60, p = 0.001) between changes in the distance to notice an intruder and rates of social play (t = 3.41, p = 0.001), as well as a significant overall relationship ($F_{1,69}$ = 12.22, p = 0.001) between changes in the distance to flee from an intruder and rates of social play (t = 3.65, p = 0.001). I further evaluated the relationship between rates of social play and changes in the distance to notice and flee from an intruder. The range of social play rates observed for the 71 juveniles in this part of the study (0.21–7.93 interactions/hour) was divided into equal thirds to assess juveniles who played at the lowest, intermediate, and highest rates. Mixed linear models with social play groupings as a fixed effect and litter as a random effect indicated that changes in the distance to notice an intruder varied significantly with social play (Fig. 4A, $F_{2,48}$ = 7.45, p = 0.002), as did the distance to flee from an intruder (Fig. 4B, $F_{2,48}$ = 5.64, p = 0.006). In particular, juveniles who played at higher rates tended to exhibit greater increases in distances to notice and flee from intruders across the play interval (Fig 4).

Among the independent variables evaluated, stepwise multiple regression indicated a significant overall relationship between ($F_{1,69}$ = 10.01, p = 0.002) between changes in the proportion of time spent vigilant during tests and age of mother (t = 3.17, p = 0.002). I further evaluated the relationship between age of mother and proportion of time spent vigilant during tests, dividing mothers into age/parity groups as above. Mixed linear models with age/parity groupings of mothers as a fixed effect and litter as a random effect indicated that the proportion of time spent vigilant during tests tended to decrease more with increasing age and experience of mothers (Fig. 5, $F_{2,48}$ = 5.82, p = 0.005).

DISCUSSION

The results of this study support the idea that juvenile social play promotes the development of adaptive behavioral responses in terms of boldness-caution in *U. beldingi*. Furthermore, this study corroborates the idea that the development of temperament in young animals is influenced by a variety of factors, including play behavior, maternal effects, and ecological constraints.

Juvenile *U. beldingi* with younger, less experienced mothers tended to notice and flee from intruders at greater distances at their first emergence from the natal burrow than juveniles with more experienced mothers. The influence of maternal care on early behavioral development has been well-documented in a variety of mammals (e.g. Johnson et al. 1996; Liu et al. 1997; Caldji et al. 1998; Birnie et al. 2013; Ainsworth et al. 2015; Czerwinski et al. 2016; Petelle et al. 2017). For example, in rats (Caldji et al. 2000a; Caldji et al. 2000b) and dogs (Guardini et al. 2016, Guardini et al. 2017), the amount of maternal care received during early infancy is known to promote composure, resilience, and adaptability of an offspring's responses to novelty and stress. In numerous mammalian species, less experienced mothers have been observed to be more neglectful and provide less proficient maternal care than more experienced mothers (e.g. O'Connor et al. 1992; Derocher and Stirling 1994; Wang and Novak 1994; Reid et al. 1995; Maestripieri and Carroll 1998; Dwyer and Lawrence 2000; Broussard et al. 2008; Pettitt et al. 2008; Zedrosser et al. 2009; Snyder et al. 2016; Cutuli et al. 2018). In the present study, the heightened caution exhibited during initial behavioral tests by juvenile *U. beldingi* with less experienced mothers may, thus, be the result of less proficient maternal care provided to them during lactation. This finding, therefore, supports the idea that maternal effects can influence the development of temperament in young animals.

At first emergence from the natal burrow, heavier juveniles exhibited greater vigilance during behavioral tests compared to juveniles with lower body mass. This may reflect the fundamental trade-off between foraging and vigilance, which are associated with competing pressures to be bold or cautious, respectfully. Heavier juveniles may have greater energy reserves (Nunes and Holekamp 1996), allowing them to place lower priority on foraging and higher priority on vigilance. By contrast, smaller juveniles may have smaller energy reserves and may need to prioritize foraging and acquisition of energy over vigilance. In a previous study by Bachman (1993), food-deprived adult *U. beldingi* were reported to express lower vigilance than food-provisioned squirrels. Larger squirrels may experience less pressure to be bold compared to smaller squirrels, who need to spend significant time and energy to foraging in order to prepare for hibernation. Larger squirrels may, therefore, be able to allocate more time and energy to vigilance rather than foraging, as was seen in the present study.

The distances at which juvenile *U. beldingi* noticed and fled from intruders as well as the proportion of time juveniles spent vigilant during intruder tests all increased over the course of the play interval. In young animals, prey species in particular, elevated caution may provide an adaptive benefit by allowing for earlier detection and evasion of potential threats. The increase in cautious responses exhibited by juveniles across development in the present study provides support for that idea. Moreover, a previous study, which looked at the development of responses exhibited by juvenile *U. beldingi* to being placed in novel testing arenas, reported an increase in boldness across the play interval (Marks et al. 2017). Combined with Marks et al. (2017), the present study supports the idea that different temperaments may be adaptive in different contexts.

Increases in the proportion of time juveniles spent vigilant across the play interval, were smallest among juveniles with older, more experienced mothers. In *U. beldingi*, more experienced mothers tend to be more vigilant and aggressive in defending their maternal territories after the young have emerged from the natal burrow compared to less experienced mothers (Nunes et al. 2000). Older, more experienced mothers may be more efficient at detecting and evicting intruders from the natal area. Consequently, juveniles with more experienced mothers may learn to relax their own vigilance and rely more on cues from their mothers, providing further support for the idea that maternal effects may contribute to the development of temperament in young animals.

Increases in the distances to notice and flee from intruders across the play interval were greatest among juvenile *U. beldingi* who engaged in social play at the highest rates. This result is consistent with the idea that play may influence the development of temperament. According to Marks et al. (2017), juvenile *U. beldingi* that engaged in the highest rates of social play also tended to express the largest increases in boldness in response to novel situations across the play interval. The combination of these two studies suggest that social play promotes the development of context-specific responses in terms of boldness-caution.

Play may promote the development of such temperaments through influences on early brain development. Pellis et al. (2010) suggested that social play may cause changes to central nervous system structure, enabling the expression of flexible and context-appropriate behaviors in adulthood. The prefrontal cortex controls the social aspects of play behavior that allow individuals to exhibit context-appropriate responses (Burleson et al. 2016). Juvenile social play causes crucial modifications to cortical neuron structure during mammalian development that promote behavioral plasticity later in life (Himmler et al. 2013; Burleson et al. 2016; Himmler et al. 2018). Thus, it is possible that such influences of juvenile social play on prefrontal cortex development in *U. beldingi* give rise to the development of the context-specific responses in terms of boldness-caution described in the present study.

I observed natural variation in play between juveniles. Social play in *U. beldingi* may be determined by a number of factors. Since social play in *U. beldingi* occurs primarily among littermates (Nunes et al. 1999), rates of social play may be influenced by litter size; e.g. individuals from single-offspring litters may experience very little to no social play interactions. Social play in *U. beldingi* may also be limited by energetic constraints, such as the size of fat reserves (Nunes et al. 2004a), food availability (Nunes et al. 1999), and fluctuations in the *U. beldingi* annual cycle (Monroy-Montemayor et al. in prep); e.g. juveniles with low body fat may engage in lower rates of social play in order to prioritize foraging and growth. In the present study, sex ratio and social play were not correlated; however, in other studies, gonadal hormones have been observed to produce sexually dimorphic patterns of social play in *U. beldingi* (Nunes et al. 1999). The combination of these variables may give rise to individual differences in play behavior, which in turn may set young animals on different trajectories for behavioral development (Lampe et al. 2017). Those that play more may be better equipped to deal with the unpredictability of an ever-changing environment.

While I examined the short-term effects of play on the development of boldness-caution in *U. beldingi*, the possible effects may also be long-term. Hurst-Hopf et al (in prep) reported correlations between juvenile social play and docility in yearling *U. beldingi*, suggesting that social play may provide long-term effects on the development of docility. Ergo, it is possible that the effects of play on boldness-caution extend beyond the juvenile period in *U. beldingi*. Since boldness-caution may be more flexible over time than docility (Petelle et al. 2013), individual variations in boldness-caution produced by social play may be more observable in long-term studies than variations in docility. A study on the long-term effects of juvenile social play on boldness-caution may provide additional support to the idea that play can have lasting effects on the development of temperament.

Overall, the results of this study provide support for the idea that social play in juvenile *U. beldingi* enhances the behavioral flexibility of juvenile squirrels, allowing them to respond appropriately when faced with a variety of stressful contexts. This study also provides support for the idea that various factors may work together to determine the development of temperament in young animals. Future investigation of the relationship between social play during the juvenile period and non-play behaviors beyond the juvenile period should aid in clarifying whether the effects of social play on temperament produce benefits that are persistent or only experienced in the juvenile stage.

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FIGURE LEGENDS

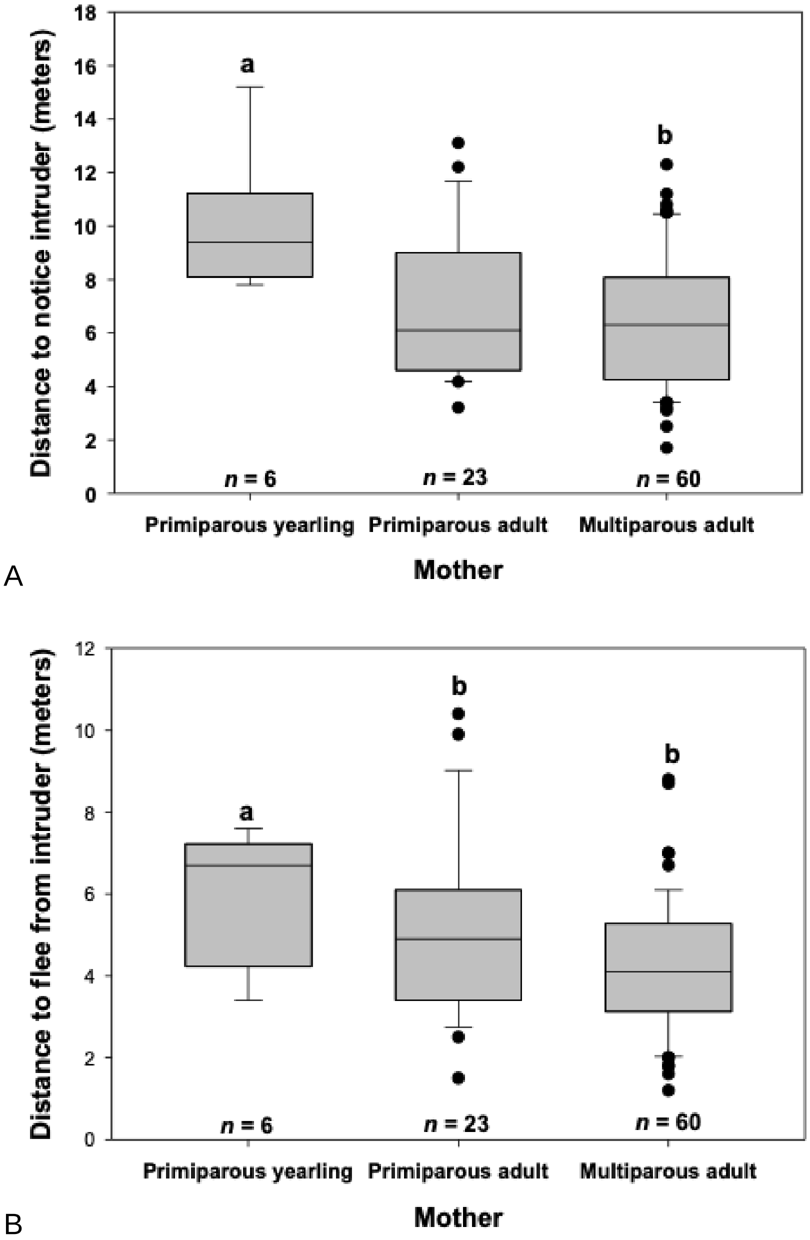

Fig. 1—Variation in distances at which juvenile *U. beldingi* (A) noticed and (B) fled from approaching intruders to the natal area at their first emergence from the natal burrow, evaluated using mixed linear models with juvenile groupings based on the age and parity of their mothers considered as a fixed effect and litter considered as a random effect.

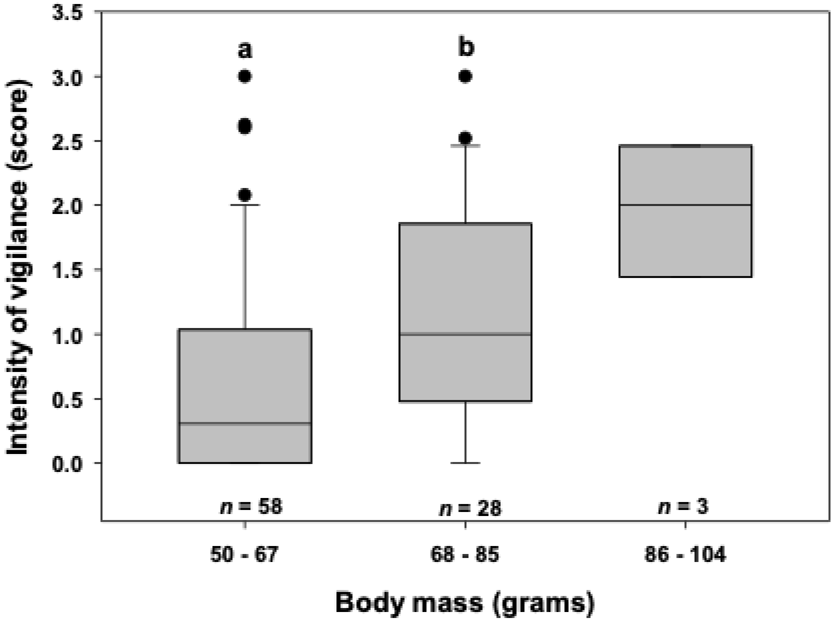

Fig. 2—Variation in intensity of vigilance displayed by juvenile *U. beldingi* during intruder tests at their first emergence from the natal burrow, evaluated using mixed linear models with juvenile groupings based on their body mass at emergence considered as a fixed effect and litter considered as a random effect.

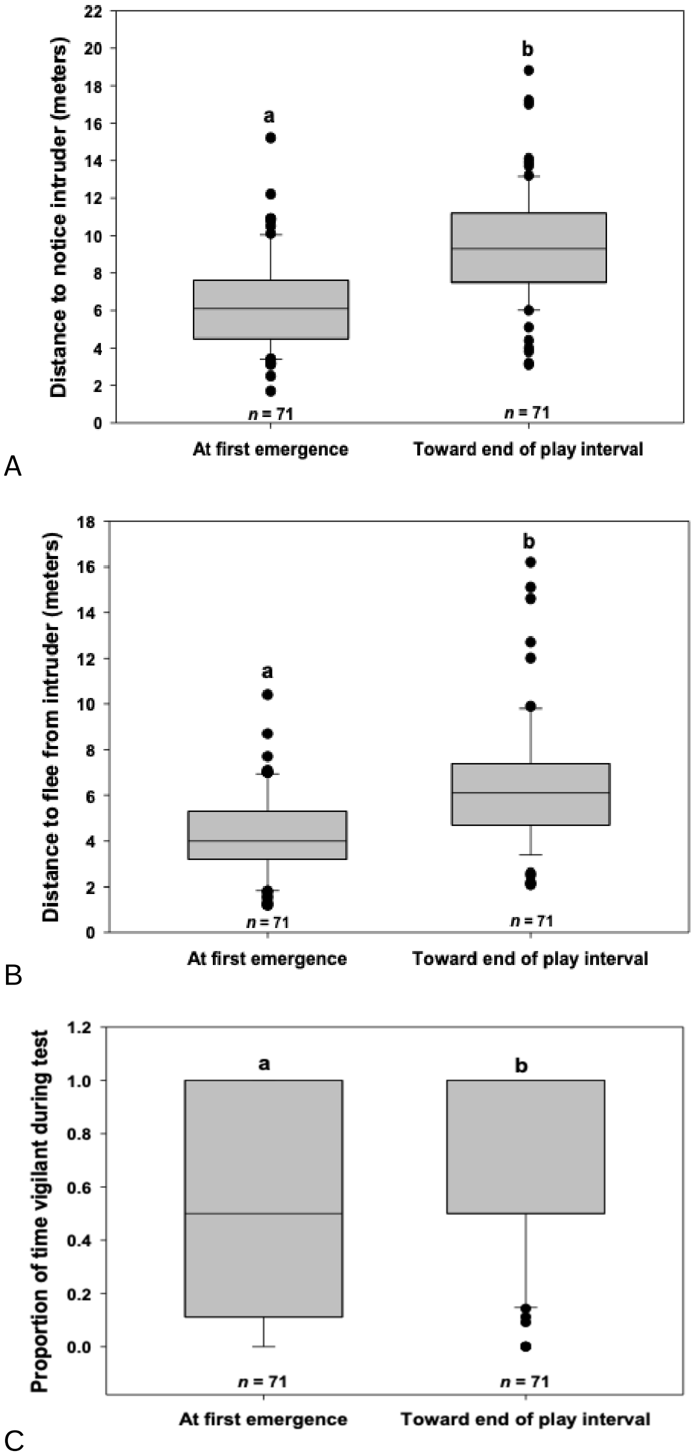

Fig. 3—Changes in distances to (A) notice and (B) flee from an intruder and in the (C) proportion of time spent vigilant during intruder tests conducted on juvenile *U. beldingi* between initial tests conducted at their first emergence from the natal burrow and re-tests conducted 2-weeks later, near the end of the juvenile play interval.

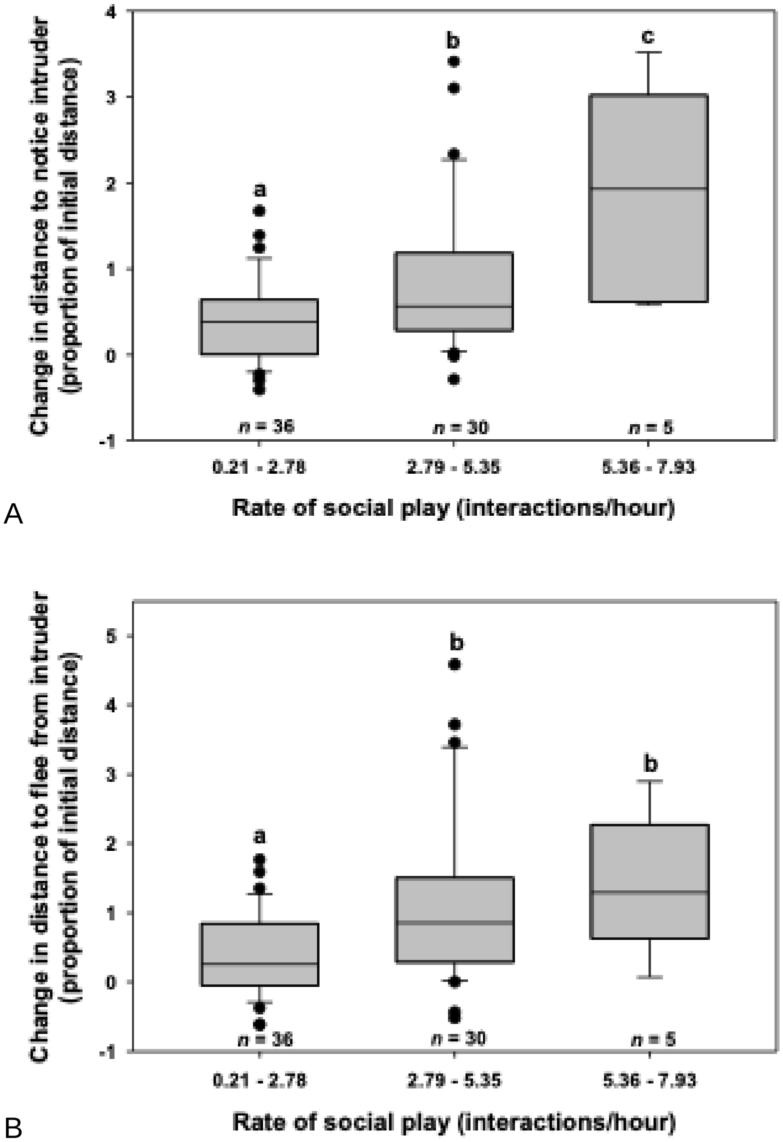

Fig. 4—Variation in changes in distances at which juvenile *U. beldingi* (A) noticed and (B) fled from approaching intruders during intruder tests conducted over the play interval, evaluated using mixed linear models with juvenile groupings based on relative rates of social play considered as a fixed effect and litter as a random effect.

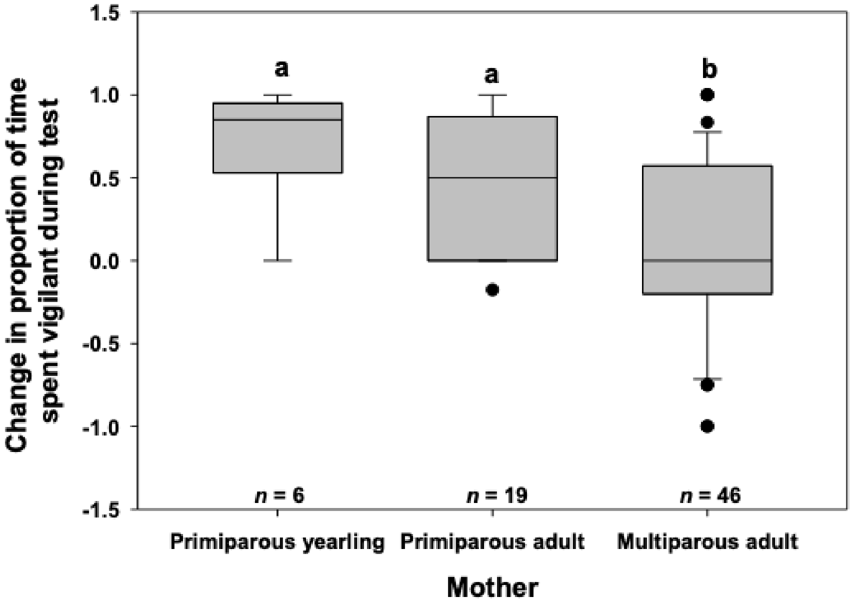

Fig. 5—Variation in changes in the proportion of time juvenile *U. beldingi* spent vigilant during intruder tests conducted over the play interval, evaluated using mixed linear models with juvenile groupings based on age and parity of their mothers considered as a fixed effect and litter as a random effect.

TABLES

Table 1.—Resting, feeding, and vigilant behavior of juvenile *U. beldingi*.

Behavior	Description
Resting	Inactive; not engaging in feeding, vigilant, or play behaviors.
Feeding	Consuming or foraging for food.
Vigilance	
Head Up	Head raised with three or four feet still in contact with the ground, while not feeding or resting.
Sit Up	Sitting back on hindquarters in a slouched position, with forelimbs off the ground, while not feeding or resting.
Post	Standing up on hind limbs which are fully extended, with back erect, while not feeding or resting.

Table 2.—Social play behavior of juvenile *U. beldingi*.

Behavior	Description
Wrestling	The juvenile faces another juvenile, typically in a ventrum-to-ventrum hold, and pecks at the other juvenile without inflicting bite wounds.
Tackling	The juvenile jumps or pounces on another juvenile, usually from a running start.
Boxing	The juvenile bats with the forepaws at another juvenile.
Chasing	The juvenile follows and pursues another juvenile for > 1 meter while both are running.
Mounting and play copulation	The juvenile climbs on the back of another juvenile and places its forepaws around the other juvenile, grasping in its mouth the other juvenile’s neck, cheek, or back, and aligning its pelvis with other juvenile’s pelvis; the juveniles typically both lie on their sides on the ground; intromission does not occur.