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Thermal tolerance affects mutualist attendance in an ant-plant protection mutualism

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Abstract

Mutualism is an often-complex interaction among multiple species, each of which may respond differently to abiotic conditions. The effects of temperature on the formation, dissolution, and success of these and other species interactions remain poorly understood. We studied the thermal ecology of the mutualism between the cactus *Ferocactus wislizeni* and its ant defenders (*Forelius pruinosus*, *Crematogaster opuntiae*, *Solenopsis aurea*, and *Solenopsis xyloni*) in the Sonoran Desert, USA. The ants are attracted to extrafloral nectar produced by the plants and in exchange protect the plants from herbivores; there is a hierarchy of mutualist effectiveness based on aggression toward herbivores. We determined the relationship between temperature and ant activity on plants, the thermal tolerance of each ant species, and ant activity in relation to the thermal environment of plants. Temperature played a role in determining which species interact as mutualists. Three of the four ant species abandoned the plants during the hottest part of the day (up to 40°C), returning when surface temperature began to decrease in the afternoon. The least effective ant mutualist, *F. pruinosus*, had a significantly higher critical thermal maximum than the other three species, was active across the entire range of plant surface temperatures observed (13.8–57.0°C), and visited plants that reached the highest temperatures. *F. pruinosus* occupied some plants full-time and invaded plants occupied by more dominant species when those species were thermally excluded. Combining data on thermal tolerance and mutualist effectiveness provides a potentially powerful tool for predicting the effects of temperature on mutualisms and mutualistic species.

Keywords

Thermal ecology; species interaction; temperature; desert; abiotic

Introduction

Organisms must respond to abiotic variation both in space, across a heterogeneous environment, and in time (Angilletta 2009). Abiotic factors, including temperature, can mediate competition among species by determining behavior, physiology, phenology, and

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life history in a way that leads to spatial and temporal resource partitioning (Magnuson *et al.* 1979; Angilletta 2009). Species differ in thermal tolerance, or the range of temperatures at which they can survive or perform (e.g. forage, reproduce, develop). Environmental heterogeneity, including abiotic factors such as temperature, promotes coexistence when species exploit resources in various ways (Palmer *et al.* 2003), such as by occupying different thermal niches (Beitinger & Magnuson 1975; Cerda *et al.* 1998a, 1998b; Bestelmeyer 2000; Buckley & Roughgarden 2005).

Many mutualisms involve multiple species, or interacting guilds (Stanton 2003). In these mutualisms, species have the potential to interact with partner species that may vary in multiple characteristics. Mutualists are quite sensitive to partner identity (e.g., due to critical differences in their phenology or behavior) and to partner quality (e.g., their effectiveness at removing a threat of attack). In protection mutualisms (those in which mutualists defend their partners from natural enemies, usually in return for a food reward; Bronstein 1998), competitive dominance may be related to mutualist quality (Ness *et al.* 2006). This is because species that are more aggressive towards their own competitors tend to be more aggressive towards their partners' enemies as well (Buckley & Gullan 1991; Madden & Young 1992; Palmer & Brody 2007; Martins 2010; Novgorodova & Gavriluk 2012), translating into increased mutualistic effectiveness of the superior competitors. These superior competitors are likely able to obtain the highest quality resources under the least stressful conditions. In a heterogeneous thermal environment, we can therefore predict that partners should be better defended at more moderate temperatures, as more effective mutualistic defenders will be present at those temperatures. Conversely, partners should be less defended at extreme high or low temperatures, as less effective mutualist partners will be abundant at those temperatures.

Mutualisms between ants and plants are common across habitats worldwide (Rico-Gray and Oliveira 2007; Oliver *et al.* 2008), which differ in the range and variability of temperatures experienced. There is abundant evidence from a variety of habitats that temperature is a major factor determining ant foraging activity (e.g. Holway *et al.* 2002; Bucy and Breed 2006; Meisel 2006; Azcarate *et al.* 2007; Dunn *et al.* 2007). In light of ants' well-studied and differing responses to temperature, they provide excellent systems for studying thermal ecology (Heil & McKey 2003). In this study, we investigate the thermal ecology of one well-studied protection mutualism involving the fishhook barrel cactus, *Ferocactus wislizeni*, and its ant mutualists in the Sonoran Desert, USA. The cactus exudes nectar from extrafloral nectaries, attracting a small assemblage of ant species. In exchange, the ants protect the plants against herbivores (primarily hemipteran insects). Typically, only one ant species occupies any given plant at any given time (at a scale of days and months; Morris *et al.* 2005, Lanan and Bronstein 2013). Ness *et al.* (2006) documented a hierarchy of mutualist effectiveness in this system based on ants' relative ability to clear herbivores from the plant.

Surface temperature often varies among plants due to their angle relative to the ground, the direction the plant is facing, whether it is in direct sun or shade, and whether or not it is bearing fruit. Thus, ants experience a heterogeneous thermal environment while foraging among plants and within a single plant (Fig. 1). This suggests that temperature should

partially determine the behavior and abundance of mutualistic ants on the plants they defend, due to the effect of temperature on foraging. Thermal tolerance could affect both ant activity (which we define as the number of ants of a given species present at a time) on an individual plant and the distribution of ant species across plants (quantified here as presence/absence). We predicted that more effective ant mutualist species interact with plants primarily at moderate temperatures, and that less effective ant mutualist species interact with plants at higher temperatures. To test these predictions, we determined (1) the relationship between temperature and ant activity on individual plants, (2) the thermal tolerance of each ant species, and (3) ant activity among plants in relation to the thermal environment of individual plants.

Materials and Methods

Study System

The fishhook barrel cactus *Ferocactus wislizeni* grows throughout the Sonoran and Chihuahuan Deserts, from Mexico into the southwestern United States, including Arizona, New Mexico, and Texas between 0 and 1500m in elevation (Turner 1995). Blooming typically coincides with the monsoonal rains, beginning in late July and continues through mid-October, with a peak in late August (McIntosh 2002). Numerous extrafloral nectaries are positioned on the aureoles at the top of the plant surrounding the fruit (see Fig. 1). Lifespan of the plants can extend beyond 40 years, and individuals produce extrafloral nectar year-round from the time they are 1-2 years old.

This study was conducted at the Desert Laboratory in Tucson, Arizona, (32.22°N, 111.01°W), a 370 ha reserve consisting of desert scrub habitat, on two study plots at which this mutualism has been extensively studied (Morris *et al.* 2005; Ness *et al.* 2006, 2009; Lanan and Bronstein 2013). Additional observations were made at a patch of similar undisturbed desert scrub habitat north of Tucson (32.62°N, 111.17°W). Field observations were made in September 2008 and September 2013. This is the time of year when *F. wislizeni* sets fruit and is potentially most vulnerable to attack by insect herbivores, which feed on the fruits and seeds. Average high temperatures during this time of year range from 29 to 36°C, with extreme temperatures above 44°C (for Tucson, AZ 1981 to 2010; National Oceanic and Atmospheric Association). It is also a relatively wet season, with average monthly rainfall of 3-5 cm.

Four native ant species make up >90% of visitors *F. wislizeni* nectaries at both study sites: *Forelius pruinosus*, *Solenopsis aurea*, *Solenopsis xyloni*, and *Crematogaster opuntiae* (Ness *et al.* 2006, Lanan, *unpublished data*). Typically, a single ant species occupies a given plant at a given time, with predictable seasonal turnover in the most abundant species across the site (Morris *et al.* 2005). The only exception to this pattern is *F. pruinosus* (discussed in Results). *Crematogaster opuntiae* is the most frequent ant (measured as the proportion of plants on which a species is active) on *F. wislizeni* during winter and spring months, whereas *S. aurea*, *S. xyloni*, and *F. pruinosus* are most frequent during summer and autumn (Morris *et al.* 2005). All four ant species occurred in high numbers on cacti during this study.

Crematogaster opuntiae colonies have been mapped in detail for one of the study sites and their occupancy of *F. wislizeni* followed for three years (Lanan and Bronstein 2013). Based on confrontation tests, sampling from individual *F. wislizeni* that are at least 10 m apart assures behavioral independence, even if ants on different plants may belong to the same large polydomous colony. Colonies maintain nest sites nearby and occupy individual cacti for extended periods of time. Long-term census data indicate that ant colonies make use of individual *F. wislizeni* as constant and reliable carbohydrate and water resources for months, with periods of rapid turnover when different ant species' activity levels are changing (J.H. Ness and W.F. Morris *unpublished data*).

Previous experiments have documented a hierarchy of mutualistic effectiveness (Ness *et al.* 2006). *Solenopsis xyloni* is more effective at removing herbivores (measured as per capita aggressiveness towards surrogate herbivores) than are *C. opuntiae* and *S. aurea*, which are equivalently effective; all three are much more effective than *F. pruinosus*. *Forelius pruinosus* is also the least dominant species in staged confrontations at baits (Lanan *et al. unpublished data*).

The most frequently observed natural enemies of the plants are hemipterans, commonly leaf-footed bugs in the genus *Narnia*, which feed on fruits and seeds. These insects (which we will subsequently term herbivores) are often observed resting between the ribs, low on the sides of *F. wislizeni* and feeding on plant tissues, including fruits, at the crown. Most observations of ants attacking *Narnia* have been made when these herbivores are on the top of the plant, near the extrafloral nectaries where the ants are foraging (G. Fitzpatrick and M. Lanan, *personal observation*).

Ant activity related to temperature

Observations of ant activity in response to temperature were made on 17-Sept-2008 and 14-Sept-2013. Both observation days were sunny with less than 10% cloud cover. Ambient air temperature was on average 2.5°C hotter on the later date; however, there was no significant difference between the ranges of plant surface temperatures measured on the two days (ANOVA, $F_{1, 227} = 0.664$, $P=0.614$). Once per hour for each plant, surface temperature and ambient temperature were recorded using an Omega 871A digital thermometer with a type K thermocouple. Surface temperature was recorded at the center of the plant to reduce position bias. This area is typically cooler than the periphery due to shading from spines and fruits (Fig. 1). At the same time, the number of ants present on the plant surface was recorded and the crown of the plant was gently tapped with a pencil to induce ants to emerge from plant crevices. Light tapping causes a short period of increased movement in the ants, but does not cause an increase in recruitment to the plant (M. Lanan, *unpublished data*). Ground temperatures were not recorded because they were extremely variable across the complex substrates found at the two sites. The location and number of *Narnia* on each plant was also noted.

On 17-Sept-2008, 6 plants were observed over 6 hr from 10 am to 4 pm. One plant was occupied by *S. aurea*, one by *C. opuntiae*, 2 by *F. pruinosus*, and 2 by *S. xyloni*. *Forelius pruinosus* also visited the plant tended by *S. aurea* for part of the observation period. On 14-Sept-2013, 16 plants were observed over 13 hr from 7 am to 8 pm. Five plants were

occupied by *S. aurea*, 3 by *C. opuntiae*, 3 by *F. pruinosus*, and 5 by *S. xyloni*. *Forelius pruinosus* also visited the 3 plants tended by *S. aurea*, 3 plants tended by *C. opuntiae*, and 4 plants tended by *S. xyloni* during parts of the observation period. Observed plants occupied by the same species were a minimum of 10 m apart to eliminate pseudoreplication of the same colony (Lanan and Bronstein 2013). The mean distance between observed plants was $38.7\text{m} \pm 7.05\text{SE}$ for *S. xyloni*, $19.2\text{m} \pm 2.9\text{SE}$ for *S. aurea*, $50.7\text{m} \pm 34.6\text{SE}$ for *C. opuntiae*, and $40.0\text{m} \pm 10.7\text{SE}$ for *F. pruinosus*.

Field study of ant thermal tolerance

Over the course of our observations, ants often abandoned the plants during the hottest period of the day and returned when the plant surface began to cool. In order to determine the temperature threshold for abandonment for each species, we used logistic regression to determine the temperature at which the probability of abandonment was 0.5. Logistic functions were fit to presence/absence data for each ant species, pooled across plants. Logistic regressions were carried out for each ant species when it was the sole visitor to a plant, as well as for *Narnia* and *F. pruinosus* when it visited plants occupied by other species. Stepwise logistic regression was used to determine whether curves for different species differed significantly from one another.

To determine whether the different ant species visited cacti that differed in their thermal profile, we compared the maximum temperature reached by plants at mid-day. We used a logistic regression model in which maximum plant temperature was the independent variable and species of ant visitor was the dependent variable.

Lab determination of ant thermal tolerance

Critical thermal maximum (CTM) of each ant species was determined in the lab using methods described by Cerda and Retana (1997, 2000). Upper thermal resistance was determined using a VWR Signature™ Heated Circulator and water bath with a 5–200°C temperature range and accuracy to $\pm 0.25^\circ\text{C}$. Trials were carried out at temperatures from 46°C (the highest temperature at which all four species have been observed in the field) up to the temperature at which more than 50% of the ants died, increasing by 2°C for each trial. Ten individual ants were used per temperature trial and placed in an open container which was suspended in the water bath. Individuals were used only once. Surface temperature of the container was allowed to equilibrate to the experimental temperature prior to trial and was measured constantly using an Omega 871A digital thermometer with Omega type K thermocouples. A total of 6 trials were carried out for *S. xyloni* (46° , 46° , 46° , 48° , 48° , 48°C), 7 for *S. aurea* (46° , 46° , 46° , 48° , 48° , 48° , 50°C), 10 for *F. pruinosus* (48° , 48° , 50° , 50° , 50° , 52° , 52° , 52° , 54° , 54°C), and 5 for *C. opuntiae* (46° , 48° , 48° , 50° , 50°C). Worker ants were collected directly from the field, then randomly selected for each CTM trial.

To determine the CTM, we used logistic regression to determine the temperature at which the probability of death was 0.5 for each species. Logistic functions were fitted to survival/death data pooled across trials and across temperatures for each ant species. Stepwise logistic regression was used to determine whether curves for different species differed

significantly from one another. To determine the relationship between body size and thermal tolerance, we measured the size of the ants after each trial, using calipers to measure total body length, from the front of the head to the tip of the gaster. We used logistic regression to test whether CTM (as determined above) was dependent on body length (pooled and averaged for the 10 ants in each trial).

Results

Ferocactus wislizeni provides a highly variable thermal environment within and among plants for foraging ants. Plants grow angled toward the southeast, such that the crown of the plant receives full sun just before noon. Thermal images (Fig. 1) clearly illustrate that the sides and crown of cacti that face the sun are the hottest regions. The relationship between plant surface temperature and ambient temperature varied with time of day (Fig. 2). Plant surface temperature peaked at 12pm, but ambient temperature continued to rise until 2pm. The maximum difference between average surface temperature and ambient temperature was 12.4°C.

Field study of ant thermal tolerance

The pattern of occupation of *F. wislizeni* by three of the ant species, *S. xyloni*, *S. aurea*, and *C. opuntiae*, was similar over the course of the day. High numbers of ants were present in the morning, decreasing until the plant was abandoned during the hottest mid-day hours, and increasing again in the evening (Fig. 3 a, b, c). In contrast, *F. pruinosus* exhibited two distinctly different patterns of behavior. First, on two plants on which it was the sole ant occupant, workers never abandoned the plant, even during the hottest hours (Fig. 3 d). Second, *F. pruinosus* also visited more than half of the plants occupied by the other ant species. On these plants, it was absent or present in only very low numbers when the more dominant species were present, but recruited large numbers of workers during the afternoon when temperatures began to decrease but the dominant ant occupants had not yet returned (Fig. 3 e).

S. xyloni, *S. aurea*, and *C. opuntiae* differed only slightly in the surface temperature at which they were likely to abandon the plant, with estimates of 40.9°C, 43.0°C, and 45.3°C respectively (Fig. 4 a, b, c; Logistic Regressions, $X^2_1=38.098$, 28.545, 24,269, all $P<0.001$, functions $Y=1/(1+e^{(-16.6+0.406x)})$, $Y=1/(1+e^{(-11.37+0.26x)})$, $Y=1/(1+e^{(-17.08+0.376x)})$ respectively). The logistic regression curves for *S. xyloni* and *S. aurea* did not differ significantly from one another, although the curve for *C. opuntiae* did differ from both *S. xyloni* and *S. aurea* (Stepwise Regression, parameter effects test $X^2_1=4.45$, $P=0.0348$). *F. pruinosus* was present across nearly the entire range of surface temperatures that were observed, and no estimate could be made for a temperature at which plants would be abandoned (Fig. 4 d; Logistic Regression, $X^2_1=0.094$, $P=0.759$, function $Y=1/(1+e^{(0.58+0.0069x)})$). Similarly, *Narnia* herbivores were occasionally observed feeding at the crown of three plants, but the range of observed surface temperatures had no effect on *Narnia* presence (Fig. 4 e; Logistic Regression, $X^2_1=1.254$, $P=0.262$, function $Y=1/(1+e^{(4.79-0.079x)})$).

Plants visited by the different ant species differed in their temperature profile. *F. pruinus* visited plants that reached the hottest maximum temperatures on average, while *S. xyloni* visited plants that reached the lowest maximum temperatures (Fig. 5). There was a significant effect of plant maximum temperature on visiting ant species identity (Multiple Logistic Regression, $X^2_3 = 11.207$, $P = 0.011$).

Lab determination of ant thermal tolerance

The temperature at which individual ants were at a 50% risk of death was estimated for each species in the lab CTM trials using logistic regression (Fig. 6). This temperature was estimated as 47.9°C for *S. xyloni* (Fig. 6 a; $X^2_1 = 47.284$, $P < 0.0001$, $Y = 1/(1 + e^{(560.3 - 11.69x)})$), 47.6°C for *S. aurea* (Fig. 6 b; $X^2_1 = 33.438$, $P < 0.0001$, $Y = 1/(1 + e^{(100.3 - 2.107x)})$), 50.1°C for *C. opuntiae* (Fig. 6 c; $X^2_1 = 14.511$, $P < 0.0001$, $Y = 1/(1 + e^{(70.54 - 1.41x)})$), and 53.4°C for *F. pruinus* (Fig. 6 d; $X^2_1 = 16.963$, $P < 0.0001$, $Y = 1/(1 + e^{(26.28 - 0.493x)})$). The logistic regression curves for *S. xyloni* and *S. aurea* did not differ significantly from one another ($X^2_1 = 0.075$, $P = 0.783$), but the curves for *F. pruinus* and *C. opuntiae* differed from one another as well as from those of *S. xyloni* and *S. aurea* (Stepwise Regression, parameter effects test, all $P < 0.001$).

Although the four ant species differed in mean body size (Table 1), body size did not appear to be a factor determining the difference in CTM between species. There was no significant relationship between thermal tolerance and body size (logistic regression, $X^2_1 = 0.009$, $P = 0.924$). *F. pruinus* ($n = 10$) was the smallest ant species and had the highest CTM, while *C. opuntiae* ($n = 6$) was the largest ant species and also had a high CTM, higher than *S. xyloni* ($n = 5$) and *S. aurea* ($n = 5$).

Discussion

Temperature is a highly variable factor in an organism's environment, both spatially and temporally. Thermal environment may affect an organism's physiology, behavior, or interactions with other organisms. While much is known about the thermal ecology of individual organisms (Angilletta 2009), research on the thermal ecology of species interactions remains scant. Ant-plant protection mutualisms, common interactions in a variety of habitats worldwide (Chamberlain & Holland 2009; Rosumek *et al.* 2009; Marazzi *et al.* 2013), provide ideal systems for testing how variation in thermal environments alters interactions. Here we have demonstrated that temperature may play an essential role in determining the structure of one desert ant-plant mutualism, by affecting which mutualistic species interacts with partners that differ in the thermal environment they occupy or the thermal niche they provide. The least effective mutualistic ant in this system tolerated and was active at higher temperatures than the more effective ant species and occupied plants that were on average hotter.

The best ant protectors of plants are aggressive and competitively dominant (Buckley & Gullan 1991; Lach *et al.* 2010). The aggressive behaviors used in competitive interactions with other ants (e.g., biting, stinging, mass recruitment) can also be directed against the enemies of species that provide them with critical commodities. Such aggressive behaviors have been demonstrated in protection mutualisms, between ants and plants (as in the system

described here; e.g., Ness *et al.* 2006; Xu & Chen 2010) as well as between ants and hemipterans (e.g., Itioka & Inoue 1999; Kaneko 2003). Thus, there may be a direct positive correlation between aggressive behavior and mutualist effectiveness in mutualistic interactions, especially those involving protection.

Forelius pruinosus is the least dominant of the four ant species that commonly defend *F. wislizeni* from herbivorous insects, as determined from staged confrontations at baits (Lanan, 2010). It is also the least effective at removing those herbivores, and as a consequence is associated with reduced fruit set (Ness *et al.* 2006). Here we have shown that *F. pruinosus* is the most thermally tolerant of the four major ant occupants, is not behaviorally limited by the range of surface temperatures plants experience during the hot part of the year, has the highest critical thermal maximum of these species, and occupies hotter plants than does the more behaviorally dominant species. *Forelius pruinosus* also exhibited two distinct behavioral strategies: it occupies some plants full-time, and invades plants occupied by more dominant species during periods of the day when those species are thermally excluded. These results suggest that plants that are on average hotter are more likely to interact with less effective mutualists, and that a rise in daily temperatures will likely increase the portion of the day when only the least effective mutualist is present. Furthermore, our results also show that the main insect herbivore in this system is not behaviorally limited by high temperature in the field.

A tradeoff has been demonstrated between thermal tolerance and behavioral dominance in certain groups of animals (Beitinger & Magnuson 1975; Cerda *et al.* 1998b; Wiescher, Pearce-Duvet & Feener 2011). Within a guild, behaviorally dominant organisms can occupy the highest quality thermal niche, the one in which performance is maximized, while less dominant organisms occupy more extreme thermal niches. This tradeoff has been shown, for example, in anole lizards (Buckley & Roughgarden 2005), bluegill sunfish (Beitinger & Magnuson 1975), and ants (Cerda *et al.* 1998a, 1998b; Bestelmeyer 2000). In the present study, the least dominant ant species relies on its exceptional thermal tolerance as an alternate strategy to access plants defended by more dominant ant species. A tradeoff between thermal tolerance and behavioral dominance (generally measured by aggressiveness) has been demonstrated in a number of ant communities, mostly in extreme thermal environments (e.g. Cerda *et al.* 1998a, Bestelmeyer 2000, Wiescher *et al.* 2011). We predict that this will have negative reproductive consequences for plants that interact with more thermally tolerant, but less dominant, and thus less effective, mutualistic ant species.

The thermal environment that these plants provide for ants is complex. Among plants, microclimatic variability, especially on the surface of the plant where the ants forage at extrafloral nectaries, is high due to location in the field (e.g., level of exposure, shading by other plants, elevation) as well as to characteristics of the plants themselves (e.g., see Fig. 1). Temperatures on the top of the cactus can be affected by the angle of the plant itself (*F. wislizeni* tend to lean, often in a southeasterly direction), by the height of the plant, and, at a within-plant scale, by shade cast by spines, flowers, and fruit. Thermally complex environments may mediate ant competition and promote coexistence by spatially segregating species based on thermal tolerance (Wittman *et al.* 2010; Wiescher *et al.* 2011). Ground surface temperatures are extremely variable at the locations studied here and likely

affect the activity of the ants around the plants, and thus on the plants themselves. For instance, ground temperature may be the factor that limits the time of arrival of *F. pruinosus* at plants tended by other ants (e.g. Fig. 2 e). While the three more aggressive ant species typically have nest entrances near (*C. opuntiae*, Lanan & Bronstein 2013) or directly below (*S. xyloni* and *S. aurea*, M. Lanan, *personal observation*) the plants, *F. pruinosus* nest in open areas and follow long trails across the ground surface to the plants (M. Lanan, *personal observation*).

This study suggests that in the extreme environment of the Sonoran Desert, ants are distributed among cacti in response to the thermal environment that the plants provide (in this case, plant surface temperature) and their critical thermal maxima. This is not surprising, as ants, especially less dominant species, in habitats that experience extreme temperatures tend to take more thermal risks by foraging closer to their thermal tolerance (Cerdeira *et al.* 1998a, Wiescher *et al.* 2011). Our results suggest that the range of temperature tolerance or critical thermal maximum (or minimum, although it was not tested here) may be important in determining which species interact in multi-species mutualisms, especially in extreme thermal environments.

It is important to point out that we have not quantified the relationship between temperature and the plants' need for defense against its enemies. Ants likely only benefit the plants in the presence of herbivores (which have been shown to impose a cost to plant fitness; Ness *et al.* 2006). If the thermal tolerance of the herbivores is below that of all four of the major ant defenders in this habitat, then their relative differences in effectiveness and upper thermal tolerance will be of less importance, because protection will not be necessary at hotter temperatures beyond the tolerance of the herbivores. However, the upper thermal tolerance of the dominant herbivore in this system, *Narnia*, is above the temperatures at which the mutualistic ant species are observed on the plant (Fitzpatrick *et al.* 2013). Therefore, there is likely a need for protection even at these high temperatures. In addition, Ness *et al.* (2006) reported higher herbivore abundance on plants tended by the least effective mutualist, *F. pruinosus*, relative to the other three species in this system. Because ants remain on plants for extended periods of time, those plants tended to by *F. pruinosus* are likely vulnerable to more fruit and seed damage even if herbivore activity decreases at higher temperatures due to their thermal tolerance. A comprehensive perspective (Bronstein & Barbosa 2002) that includes activity, abundance, and frequency of herbivores across the temperature gradient will be necessary to fully understand the thermal ecology of the mutualism.

Thermal ecology studies to date have largely ignored species interactions, especially mutualism. The studies that do relate temperature to mutualism are limited to a few charismatic, threatened systems (especially corals and pollinators; Walther *et al.* 2002; Hoegh-Guldberg *et al.* 2007, Tylianakis *et al.* 2008). In the protection mutualism described here, the least effective ant species may be avoiding competition by occupying an extreme thermal niche. Thus, rising temperatures could decrease mutualistic benefit to ant-defended plants by favoring thermally tolerant but competitively and mutualistically inferior ants. Future work should investigate how thermal ecology affects partner distribution and frequency in multi-species mutualisms and how that affects the benefits received by the mutualist partners. Even small qualitative differences among mutualist partners can have

evolutionary consequences over many generations (Kiers *et al.* 2010). It is likely that there are broad and predictable consequences for ant-plant mutualisms due to their cosmopolitan distribution and the consistent patterns in ant behaviors and response to temperature. Given the ubiquity of mutualisms and their centrality in key ecosystem functions (Kiers *et al.* 2010), this issue is of particular concern in a world expected to grow significantly warmer in the next few decades (Davis *et al.* 1998; Tylianakis *et al.* 2008; Traill *et al.* 2010).

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Fig. 1.

These images show a) *C. opuntiae* foraging at extrafloral nectaries on the surface of a fishhook barrel cactus, b) a typical fishhook barrel cactus (size, angle, direction, and spine cover are highly variable among plants) and c) a thermal image of the same plant taken with a FLIR T-300 thermal imaging camera. The color scale is shown on the right; dark blue regions are the coolest (27.7°C) and light yellow the hottest (48.5°C). This photo was taken in the field (Tumamoc Hill Desert Laboratory, Arizona) in late October 2011

Fig. 2. Ambient temperature (solid line) and plant surface temperature (dashed line) on Sept. 14, 2013. Error bars indicate standard error

Fig. 3.

The number of ants present over time on Sept. 14, 2013 is shown for a) five plants with *S. xyloni*, b) five plants with *S. aurea*, c) three plants with *C. opuntiae*, and d) three plants with *F. pruinosus* (solid lines). The number of *F. pruinosus* visiting ten cacti that were also visited by the other ant species is shown in e). Average surface temperatures of the plants in each figure are represented by a dashed line, with error bars indicating standard error of the mean

Fig. 4.

The probability of ants (or bugs) being present in response to plant surface temperature for a) *S. xyloni*, b) *S. aurea*, c) *C. opuntiae*, d) *F. pruinosus*, and e) bugs in the genus *Narnia*. Presence/absence data is represented by histograms. The temperature at which there is an equal chance of ants being present or absent is indicated in graphs a-c. Logistic regression functions are a) $Y=1/(1+e^{(-16.6+0.406x)})$ ($X^2_1=38.09$, $p<0.0001$), b) $Y=1/(1+e^{(-11.37+0.26x)})$ ($X^2_1=28.55$, $p<0.0001$), c) $Y=1/(1+e^{(-17.08+0.376x)})$ ($X^2_1=24.27$, $p<0.0001$), d) $Y=1/(1+e^{(0.58+0.0069x)})$ ($X^2_1=0.093$, $p=0.759$), e) $Y=1/(1+e^{(4.79-0.079x)})$ ($X^2_1=1.254$, $p=0.26$)

Fig. 5.

The maximum temperature reached by plants visited by each of the four ant species.

Fig. 6.

The critical thermal maximum (CTM) for each of the four ant species, estimated as the temperature at which ants have a 0.5 probability of dying. Death/survival data is represented by histograms, and the temperature at which there is a 0.5 probability of dying is noted on each curve. Logistic regression functions are a) $Y=1/(1+e^{(560.3-11.69x)})$, $X^2_1=47.284$, $P<0.0001$, b) $Y=1/(1+e^{(100.3-2.107x)})$, $X^2_1=33.438$, $P<0.0001$, c) $Y=1/(1+e^{(70.54-1.41x)})$, $X^2_1=14.511$, $P<0.0001$, d) $Y=1/(1+e^{(26.28-0.493x)})$, $X^2_1=16.963$, $P<0.0001$

Table 1

Summary of the thermal ecology of the four common ant mutualists that interact with *Ferocactus wislizeni* at the study site. Mutualist effectiveness rank is based on the hierarchy as determined by Ness *et al.* (2006). Mean temperature while active and mean plant surface temperature were calculated from measurements in the field. Plant abandonment by ant species was calculated as the temperature at which the probability of abandonment was 0.5. Critical thermal maximum values reported here were calculated as the mean temperatures at which at least 50% of the individual ants tested died, using data from laboratory experiments with field-caught ants.

Ant Species	Mutualist effectiveness rank (Ness <i>et al.</i> , 2006)	Temperature at which ants abandon plant °C	Mean temperature at which 50% of ants die °C	Body size length mm (SE)	Average max surface temperature of visited plants °C (SE)
<i>S. xyloni</i>	High	40.9	47.9	1.19 (0.20)	48.8 (1.4)
<i>S. aurea</i>	Moderate	43.0	47.6	1.17 (0.20)	52.7 (1.1)
<i>C. opuntiae</i>	Moderate	45.3	50.1	2.72 (0.18)	52.4 (0.6)
<i>F. pruinosa</i>	Low	Not within observed temp range	53.4	0.72 (0.13)	54.5 (1.2)