# Morphological characteristics of ant assemblages (Hymenoptera: Formicidae) differ among contrasting biomes

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## Abstract



The environment is thought to strongly shape the ecology and evolution of species. Similar environments may cause species to look the same or converge upon particular traits. Dissimilar environments can cause species to look different or to diverge in their traits. These ideas have been explored at the single species level or within restricted geographic areas for ants. We ask whether the signature of environmental filtering can be detected within ant faunas at the biogeographic scale. We quantify differences in the morphological traits of ant faunas between two contrasting biomes: a rainforest in Costa Rica and a desert in Iran. Lists of species from each habitat type were compiled, and measurements of ten functional traits were taken from scaled images. Body size, relative femur length, and tibia length were significantly smaller in Costa Rica suggesting species in more complex environments are smaller in response to environmental complexity. Relative eye width was also smaller in Costa Rica, and individuals were lighter in colour. Eye size and body pigmentation showed differences which may be a result of altered foraging patterns or protection against UV-B irradiance. The Costa Rican fauna also had a much wider trait space (higher functional richness), and Iranian species occupied a narrow range in terms of mandible and leg size suggesting species invest less in foraging specialism in unproductive environments. Significant differences in morphological traits and their degree of overlap can therefore be observed in ant faunas occupying contrasting biomes. We suggest that this is due to the environment influencing the trait composition of these faunas at biogeographic scales.

Key words: Ants, biomes, Costa Rica, functional traits, Iran, morphology.

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### Introduction

Environmental conditions play a critical role in shaping the ecology and evolution of species. Species must be able to cope with the prevailing temperature, rainfall, humidity, habitat structural complexity and disturbance regimes at a location if they are to exist there. This idea forms the basis of community assembly theory - how is it that species come to exist in the places and combinations that they do (WEIHER & al. 2011)? It is thought that species must be able to pass through a series of hierarchical abiotic and biotic filters if they are to survive at a site. The first filters a species must pass through are abiotic (temperature, rainfall, etc.). This suggests two related predictions. Firstly, a given environment should force species to converge on similar adaptive strategies via their morphological, physiological or behavioural traits; the trait composition of tropical tree communities appears to be constrained in this way (SWENSON & al. 2012). Secondly, different abiotic

challenges will cause species to display divergent adaptive traits between different environments. Evidence of this can be seen in the different life history strategies of bee communities living at high and low elevations (HOISS & al. 2012). High elevation bee communities have a much greater proportion of large bodied and ground nesting species that are able to cope with the harsher conditions.

In this study, we use morphology as a proxy for ecology and ask whether the environment can control what assemblages look like across biogeographic scales for an important and dominant insect group – the ants. Ants are abundant, diverse and dominant across all terrestrial biomes except the poles (DUNN & al. 2009). They make up to 15 -20% of all terrestrial animal biomass (SCHULTZ 2000) and up to 50% of invertebrate individuals in the tropics (C.L. Parr, unpubl.). Previous studies have explored the relationship between ant morphology, local environment, and life

Tab. 1: Selected morphological traits measured in each ant assemblage, traits analysed and their suggested functional significance.

Characteristic	Trait measured	Trait analysed	Functional significance		
Head size	Head width	Head width	Head size may vary allometrically with body size, head size may also be linked to predatory strategies, with wider heads allow-		
	Head length	Head length	ing for larger mandibles and therefore larger prey (KASPA 1993, SARTY & al. 2006).		
Clypeus	Clypeus length	Relative clypeus length	Modified clypeus is more common in species that rely on liquid food (EISNER 1957, DAVIDSON & al. 2004).		
Mandible size	Mandible length	Relative mandible size (Man- dible length / Head width)	Predation is linked to mandible size with larger mandibles allow- ing larger prey (WEISER & KASPARI 2006).		
Eye position	Interocular distance	Relative interocular dist- ance; Relative eye position = (Head width – Interocular distance) / Head length	More dorsally positioned eyes (bigger interocular distance) a characteristic of visual predators (FOWLER & al. 1991).		
Eye size	Eye width	Relative eye width	Wider eyes may be found in more predatory species, or alte natively may depend on foraging period, with nocturnal or lo light level foragers having bigger eyes (WEISER & KASPAR 2006, NARENDRA & al. 2013).		
Leg length	Hind tibia length	Relative tibia length	Leg length size increases locomotion speed in simple habitats;		
	Hind femur length	Relative femur length	where habitat is complexed smaller leg length allows exploita- tion of crevices and manoeuvrability (SARTY & al. 2006, GIBB & PARR 2010, 2013).		
Mesosoma	Weber's length	Weber's length	Indicative of overall body size and often linked to resource use		
	Pronotum width	Relative pronotum width	(Kaspari & Weiser 1999).		
Colour	N / A	Lightness index (v)	Pigmentation may be an adaptation to thermoregulation and exposure to solar radiation with lighter individuals occurring where temperatures are higher (WILLMER & UNWIN 1981).		

history, shedding light on potential environmental filters and the ways in which ants use their morphology.

For example, a number of studies have investigated the link between body size, leg length and habitat complexity - an idea called the size grain hypothesis. This hypothesis suggests that ants in complex, interstitial habitats should have relatively small legs in order to move around efficiently. This limitation is removed in simple, planar environments and relatively long legs are favoured. Evidence supporting this has been repeatedly found (KASPARI & WEI-SER 1999, SARTY & al. 2006, GIBB & PARR 2010, 2013). There are also links expected between diet and morphology. Predacious ants tend to have small, laterally positioned eyes (WEISER & KASPARI 2006, GIBB & al. 2014). Despite these studies, however, there has not yet been a comparison of the relationship between ant morphology and the environment across broad geographical scales. Do these same local relationships - indicative of environmental filters hold at the biogeographic scale?

We compare ant morphologies from assemblages in two contrasting biomes: deserts and rainforests. Tropical rainforests represent a highly productive environment. They have relatively consistent temperatures, high moisture, low light levels and a high diversity of plants and animals. The constant layer of leaf-litter supports extensive diversity which may enable a range of survival strategies (GREEN-SLADE 1983, SILVA & BRANDAO 2010). In contrast, deserts tend to have low resource availability and low surface complexity, highly variable temperatures (from below freezing to more than 40 °C) and low water availability (EDGELL 2006). These factors will likely provide a smaller range of potential niches under more severe conditions (BROWN & al. 1979).

Given the large differences in abiotic and biotic conditions between these two biomes we expect there to be morphological differences between the ant faunas found in them. Overall, we expect there to be a greater range of morphologies present in the rainforest due to the greater number of opportunities that this environment offers. We also predict that there will be differences between individual morphological traits based on specific hypotheses:

- 1. We predict that ants in desert habitats will be larger and have relatively longer legs – in accordance with the size grain hypothesis (KASPARI & WEISER 1999).
- 2. We expect that eye sizes will be smaller in the rainforest because many rainforest dwelling species forage within the leaf litter layer (FISHER 1999b), which is nearly always dark. Consequently, they may be selected to have a smaller eye size (WEISER & KASPARI 2006). We call this the light level-eye size hypothesis.
- 3. We predict that desert ants will be lighter in colour than rainforest ants because of the necessity of reflecting the desert sun. One prediction of the thermal melanism hypothesis is that lighter coloured ecotherms should occupy hotter environments to reflect incoming radiation and avoid overheating (CLUSELLA-TRULLAS & al. 2008, Tab. 1). Single species examples exist of desert ants adapting to reflect light in order to thermoregulate (SHI & al. 2015).
- 4. Due to a wider range of available foraging options, we predict that there will be more specialist predator species in rainforest habitats, manifested through larger

mandible sizes on average (DAVIDSON 1978, Tab. 1). In contrast, desert ants will be largely omnivorous; omnivory and low levels of foraging specialism in arid biomes have been documented for various taxa (e.g., ants, mammals and birds) and may be the most successful strategy in unproductive, highly unpredictable biomes (MORTON & BAYNES 1985). Consequently, the Iranian fauna will have smaller mandibles on average. In addition, this will result in the occupation of a greater range of multivariate trait space for Costa Rica. We call this the foraging specialism hypothesis.

We collected trait data from representative lists of ant species from Costa Rica and Iran. These two regions have welldocumented faunas, high- resolution images of ants available and represent two contrasting biomes. Ideally, an interbiome comparison would be performed within the same biogeographic region. In this case, we are limited by data availability. At a global scale, it is unknown how the different evolutionary histories of ant faunas have affected their ecology.

## Methods Study sites

We measured ant species from the Central Persian and Southern Nubo-Sindian desert regions of Iran, and those known to occur in the tropical rainforest region of Costa Rica. Costa Rica is a small country compared to Iran (UN-ITED NATIONS STATISTICS DIVISION 2012). Approximately 51% of the land-mass in Costa Rica is covered in tropical forest (LINDQUIST & al. 2012) and despite its relatively small size; the country is home to an estimated 4.5% of global biodiversity, making it a biodiversity hotspot (KOHL-MANN & al. 2010). The desert biomes of Iran are drier (annual precipitation = 122 - 127 mm) and cooler (average annual temperature = 17.1 °C) than the rainforests of Costa Rica (annual precipitation = 4000 mm, annual average temperature ~ 25 °C) (HESHMATI 2007, WHITFIELD & al. 2007, PAKNIA & PFEIFFER 2011b). The seasonal variation in arid biomes, such as deserts, is more extreme than that in rainforests. Rainforests experience a reduced seasonal and diurnal temperature fluctuation (HESHMATI 2007, WHITFIELD & al. 2007). Rainforest biomes support a complex surface environment of leaf litter, deadwood and detritus which varies in depth with season (WOOD & al. 2005). This contrasts with arid biomes that have a planar and sparse environment with little vegetation (PAKNIA & PFEIFFER 2011b).

# **Species lists**

A list of rainforest dwelling species from Costa Rica was compiled by combining a comprehensive list from LON-GINO & al. (2002) with additional species from the same location catalogued online (academic.evergreen.edu). For Iran, a list of species was compiled from two studies (PAK-NIA & PFEIFFER 2011a, b). The first provided a comprehensive list of all species found in Iran, whilst the second was used to determine which species lived in the desert. All species names were validated and amended if necessary using the antcat.org website.

# Measurements

Measurements were taken of morphological traits related to life history (Appendix S1, as digital supplementary material to this article, at the journal's web pages); Table 1 details the measurements taken and the functional significance of each trait. This was done using scaled images from www. antweb.org, www.antbase.net, antwiki.net, lucidcentral.org, and academic.evergreen.edu (Appendix S2). Measurements were only taken for worker or minor worker individuals. Six specimens per species were measured where possible. For the majority of specimens an image was available in head, profile and dorsal views. The ImageJ measurement software (version 1.47) was used to obtain measurements of ten morphological traits (where possible). Trait measurements were standardised by head length. Head length was used to standardise the traits (trait value / head length) as this was missing from the dataset the least frequently and is highly correlated with overall body size (WEISER & KASPARI 2006). This was for all traits except relative mandible size, which was standardised instead by head width. This was done in line with previously published work (BISHOP & al. 2015) and is intended to express the size of the mandible relative to what feasibly may fit inside the mouthparts. Head width was deemed a better measure than head length in this instance. Colour values were assessed for the head, mesosoma and gaster of each specimen based on a colour wheel developed for the Global Ant Database (Appendix S3). Colour values were converted to the HSV colour model which controls for light intensity variance (RAJA & al. 1997), with analysis based on lightness value v, where black is 0 and white is 1. Measurable images were available for 64.5% of the Costa Rican species and 100% of the Iranian species (Appendices S4 - S7).

# Statistical methods

**Univariate:** We compared each trait across the two biomes using a bootstrapped t-test with permuted p-values. Bootstrapping allows us to circumvent the problem of differences in regional species pool size by resampling from the larger Costa Rican fauna with replacement (in this case 999 replications) (XING & al. 2014). The p-values were corrected for multiple testing using a Bonferroni adjustment (WESTFALL & WOLFINGER 1997) from the *coin* package in R (ZEILEIS & al. 2008).

Multivariate: A convex hull volume estimation method was used to describe the minimum multidimensional volume that includes all species in each of the ant assemblages (CORNWELL & al. 2006). From this, the multidimensional overlap between the two biomes was calculated. Not all species had a complete list of traits due to missing images or obscured appendages. Volume estimation methods, however, may struggle with incomplete datasets (LA-LIBERTÉ & LEGENDRE 2010). To counter this, we estimated the missing trait values based on those of the other species in the dataset using multiple imputation with chained equations (MICE version 2.22 in R) (BUUREN & GROOTHUIS-OUDSHOORN 2011). This method has a relatively small error compared to others. In addition, alternative solutions such as removing incomplete records can lead to bias, reduced statistical power and an incomplete understanding of the ecology in question (PENONE & al. 2014). Principle coordinates analysis (PCoA) was conducted on six traits from the dataset (including the imputed values) for the analysed traits shown in Table 2. The traits were relative eye width and position, relative femur length, relative mandible length and head length.



Fig. 1: Trait value differences by region for statistically significant relative traits, showing lower values for Costa Rica across all size traits, as well as lighter pigmentation across individuals. A plot for relative mandible length is also included for illustration, although for this trait there was no significant difference.

Tab. 2: Bootstrapped estimated differences of traits between biomes including standard deviation of the difference, 95% confidence intervals, the test statistic Z and permuted p- values. Statistically significant results are shown in bold for Weber's length, relative eye width, relative femur length, relative tibia length and for colour, indicated by the lightness v.

Trait	Estimated dif- ference between biomes	SD	95% Lower CI	95% Upper CI	Z	р
Weber's length	-0.71	0.23	-1.16	-0.27	-3.61	< 0.01
Relative mandible length	0.03	0.02	-0.01	0.07	0.65	0.66
Relative clypeus length	0.02	0.01	0.00	0.03	1.25	0.21
Relative eye width	-0.06	0.01	-0.07	-0.04	-4.71	< 0.01
Relative femur length	-0.30	0.07	-0.43	-0.17	-3.67	< 0.01
Relative interocular distance	-0.02	0.03	-0.07	0.03	-0.72	0.474
Relative pronotum width	0.07	0.02	0.02	0.11	1.53	0.126
Relative tibia length	-0.34	0.08	-0.50	-0.17	-4.91	< 0.01
Relative eye position	-0.03	0.02	-0.07	0.00	-1.48	0.138
Lightness index (v)	0.23	0.05	0.14	0.33	3.93	< 0.01

These were chosen a priori as potentially relating to distinct functional adaptations (Tab. 1) and also because they were the most complete prior to the MICE procedure. The PCoA produced six synthetic axes that were used to compute the convex hull. A PCoA was used as it has become the standard ordination method in the functional traits literature (e.g., BISHOP & al. 2015, GRIFFITHS & al. 2015) and easily allows a set of orthogonal traits to be created using a variety of distance matrices (VILLÉGER & al. 2008). A similar bootstrapping procedure as above was used to control for the much greater number of species in Costa Rica. The Costa Rican dataset was subsampled to

25 species 999 times without replacement and the functional volume recalculated in order to generate confidence intervals. These analyses used the *FD* and *betapart* packages in R (LALIBERTÉ & LEGENDRE 2010, BASELGA & ORME 2012, LALIBERTÉ & al. 2014).

**Data subsets:** The same analyses (univariate and multivariate) were also performed on a subset of the data where species in Costa Rica that were known to occur in the canopy were excluded (Appendix S8). We did this to ensure that there was no bias introduced by the Costa Rican arboreal fauna since the Iranian desert fauna is epigaeic.



Fig. 2: Biplot outlining the morphological trait space for the first two PCoA axes (those showing the most variation) for all species combined. The traditional x and y axes describe the assigned trait scores from the analysis, while the right hand and top axes describe the correlation of synthetic trait scores to the original traits. These correlations are shown with red labels and arrows, depicting relative eye width (EW), relative eye position (EP), relative femur length (FL), head length (HL), relative mandible length (ML), and lightness (Li) positions.

Tab. 3: Eigenvalues and trait loadings of a PCoA, representing the morphology of both Costa Rican and Iranian ant communities. Eigenvalues quantify the role of each axis in explaining the morphological variation. Trait loadings are inferred for the traits where the main variation emerges, indicating the strength and direction of correlation of the traits with each axis.

Eigenvalues	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalue	711.67	381.34	254.67	204.95
Relative eigenvalue	0.39	0.21	0.14	0.11
Cumulative eigenvalue	0.39	0.60	0.74	0.85
Trait loadings				
Head length	-0.42	-0.49	0.29	0.36
Relative femur length	-0.45	-0.32	-0.36	-0.14
Relative eye position	-0.48	-0.06	-0.42	0.22
Relative mandible size	0.20	-0.69	0.13	-0.60
Relative eye width	-0.43	0.39	-0.10	-0.66
Lightness index (v)	0.41	-0.17	-0.76	0.07

### Results

Functional trait measurements were taken from the available species in each list used. This amounted to 25 species and 3 specimens per species on average for Iran and 275 species and 2 specimens per species on average for Costa Rica (Tab. 3, Appendix S4).

## Univariate analysis

The bootstrapped t-tests showed significant differences between the biomes for five traits: Weber's length, relative eye width, relative femur length, relative tibia length and lightness (Tab. 2). Trait values for Costa Rica were consistently smaller than those in Iran. Iranian ants have relatively larger legs, eyes and overall body size compared to Costa Rica. Costa Rican ants were lighter in colour than those in Iran (Fig. 1). There were no significant differences between the biomes for the remaining traits (Tab. 2).

#### Multivariate analysis

The first two PCoA axes represented ~ 60% of the morphological trait variation in the dataset, with 40% of the variation being described by axis 1 (Tab. 3). The resultant biplot is shown in Figure 2. From the trait loadings (Tab. 3), we can interpret axis 1 as a gradient of ecologies relating to habitat structure and openness, temperature regime and possibly predation. Negative scores on this axis described species with large heads, relatively long legs, darker pigmentation, and dorsally positioned eyes. Leg length and head size are traits associated with foraging in planar, open and less complex habitats (PARR & al. 2003, GIBB & PARR 2013, BISHOP & al. 2015). Dorsally positioned eyes are often associated with predatory behaviours (SILVA & BRANDAO 2010), while darker pigmentation may be associated with lower ambient temperatures (CLUSELLA-TRULLAS & al. 2007, 2008). The opposite was true of positively scoring traits on this axis, indicating individuals were smaller sized and had smaller, more laterally positioned eyes and lighter pigmentation: Traits largely related to foraging in more dense and complex habitats (GIBB & PARR 2013), reflectance of heat in higher temperatures and potentially less predatory behaviours.

Axis 2 represents a gradient of predatory specialism, negative scores represent species with small heads, relatively short legs and relatively short mandibles; traits which largely relate to more generalised foraging. Negative scores on axis 2 represent species with larger mandibles, longer legs and large heads; traits which in the main part seem to correlate with predatory specialisation (SILVA & BRAN-DAO 2010) although longer leg length does not tend to be characteristic of predatory specialism (WEISER & KASPARI 2006).

The convex hull (Fig. 3) volume of Costa Rica is much larger than that for Iran (11.52 for Iran, with a mean  $\pm$  standard error bootstrapped volume of 29.86  $\pm$  0.39 for Costa Rica). The average  $\pm$  standard error (following bootstrapping) shared volume was 3.65  $\pm$  0.043. Rainforest species had a much larger volume of unique traits, almost entirely encompassing the desert-dwelling fauna.

With axis 1 representing traits within the Costa Rican volume associated with habitat structure (with larger individuals being represented by negative values), there appears to be substantially more small rainforest species represented at the positive end of the axis, with Iranian desert species tending to occur at the negative end with larger body and leg size (Fig. 3). This matches our univariate results. For the predation gradient of axis 2, desert dwel-



Fig. 3: Convex hull projection of functional trait space for Costa Rican rainforest community ants (larger dashed black line) and Iranian desert community ants (smaller dashed red line); species for each region are represented by the same colours. Axis 1 defines a gradient of traits responding to habitat structure (negative: long leg, large body, large dorsal eyes; suited to planar environments. Positive: shorter leg, small body, small laterally positioned eyes; suited to complex environments). Axis 2 defines a gradient of traits responding to predatory specialism (negative: small head and leg length, small relative mandible size; generalist forager; positive: large head and leg length, large relative mandible size; predatory specialist). The right hand and top axes describe the correlation of synthetic trait scores to the original traits.

ling species appear to occupy the central portion of trait space, suggesting they are less specialised than the Costa Rica ants.

#### **Data subsets**

The same qualitative results were found when excluding ants from the Costa Rican canopy (Appendix S9). In the univariate analysis Weber's length, relative eye width, relative femur length, relative tibia length and lightness were significantly different. In the multivariate analysis the volume of trait space occupied by the Costa Rican fauna still dwarfed that of the Iranian fauna (Iran = 11.52, Costa Rica mean  $\pm$  standard error = 21.56  $\pm$  0.29) and the overlap was similar in magnitude to the full analysis (2  $\pm$  0.03). The removal of the Costa Rican canopy fauna did not affect the direction of magnitude of our results.

#### Discussion

For the first time, we show that there are broad differences in the morphology of ant faunas that occupy different biomes and geographic areas. We find that ants in the deserts of Iran are, on average, larger with longer legs, bigger eyes and darker cuticle colours than the ants of Costa Rica (Tab. 2, Fig. 1). We also see that the Costa Rican ant fauna occupies a much larger morphological space which almost entirely encompasses that of Iran. The Iranian ants have relatively generalist combinations of traits, whilst the Costa Rican ants have representatives of extreme and generalist morphologies. We suspect that these differences in univariate and multivariate trait descriptions are a consequence of the strongly contrasting environments of Costa Rica and Iran.

We find biogeographical support for the size grain hypothesis in ants. Our univariate analysis showed differences in body size and relative leg length (Tab. 2) between the two biomes. The size grain hypothesis predicts that there is a threshold at which large legs no longer provide efficient movement to smaller organisms, and that while increased leg size is advantageous in simple environments (speed and efficiency), the cost of restricted access to interstices outweighs the benefits as environments become more complex (KASPARI & WEISER 1999). The implication of this is that ants foraging in more planar environments would be larger with greater relative leg length (PARR & al. 2003, FARJI-BRENER & al. 2004, SARTY & al. 2006, WEISER & KASPARI 2006, GIBB & PARR 2010, WIESCHER & al. 2012, GIBB & PARR 2013). Our finding of larger and longer-legged ants in the deserts of Iran supports this idea and our initial prediction.

Our data also find support for the light level-eye size hypothesis. We predicted that eye sizes would be smaller in Costa Rica as a consequence of many species foraging within the dark environment of the leaf litter (FISHER 1999a, WEISER & KASPARI 2006). We find significantly smaller eye sizes, on average, in Costa Rica but this difference is relatively small (Tab. 2). A more detailed analysis of species' microhabitats may be able to directly link eye sizes to lower light conditions.

Contrary to our prediction, we found that ants in Iran were darker, on average, than those in Costa Rica. We expected that ants would be lighter in colouration in order to reflect more radiation (e.g., SHI & al. 2015). Difference in ant colour (pigmentation) may be related to differences in the thermal environment mediated by a thermoregulatory mechanism such as that proposed by the thermal melanism hypothesis (CLUSELLA-TRULLAS & al. 2007). The thermal melanism hypothesis suggests that darker pigmentation is an advantage in lower temperatures as low body surface reflectance allows faster heating (CLUSELLA-TRULLAS & al. 2007, 2008). It may be that the desert ants of Iran are avoiding peak temperatures and foraging seasonally (during the day in cooler months) or at the cooler parts of the day in summer. This could explain their darker colouration compared to the Costa Rican ants. Under this idea, desert ants could actually encounter lower ambient temperatures than those in the rainforest and have adopted a darker body colour to gain heat more effectively. This idea, however, is unlikely to explain the lighter colouration of the rainforest ants. The thermal melanism hypothesis relies on solar radiation reaching the organisms bodies. Under the canopy of the Costa Rican rainforest, solar radiation will be limited. We suggest, therefore, the thermoregulatory benefits of being dark are being used by the Iranian ants but are not applicable to the Costa Rican fauna. In Costa Rica the variation in lightness values around 0.5 (on a scale of 0 - 1) may represent adaptations to completely different selective pressures, such as disease resistance (WILSON & al. 2001), or could simply be the result of historical artefacts.

An alternative, and not mutually exclusive hypothesis, is that ants in Iran may be darker as melanin helps protect

against harmful UV-B irradiation (HODKINSON 2005). In areas where there are high levels of UV-B, organisms have been observed to have higher levels of melanin (CARO 2005, BASTIDE & al. 2014, KOSKI & ASHMAN 2015). It may also be that this protective role explains the differences in the colour of ants between the desert and rainforest biomes. Under the forest canopy, ants are unlikely to require UV-B protection. The opposite is likely to be true in the desert. Therefore, a protective role of UV-B may provide a better explanation of our observed differences in ant colour between biomes. Our data are unable to tease apart these competing hypotheses (thermal melanism versus UV-B protection), yet preliminary data from other studies (T.R. Bishop, M.P. Robertson, H. Gibb, B.J. van Rensburg, B. Braschler, S.L. Chown, S.H. Foord, C.T. Munyai, I. Okey, P.G. Tshivhandekano, V. Werenkraut & C.L. Parr, unpubl.) suggest varying UV-B irradiance, ambient temperatures and melanin levels tied with ant assemblage information may be able to provide a clearer picture as to the relative roles of these two hypotheses.

Initially, we predicted that due to the greater opportunities present in the Costa Rican rainforest there would be more specialist predators living there. We termed this the foraging specialism hypothesis. We expected this to be indicated by longer mandible lengths. We did not find this in our univariate analysis (Tab. 2). Either there is no difference between the biomes in the numbers of predatory specialists or our use of mandible length as a proxy for this kind of behaviour is inappropriate. We do find differences in the multivariate space occupied by the biomes, however. This suggests that there are differences in the proportions of predatory specialists in each biome (Fig. 3). Costa Rica occupies a much wider range of axis 2 (which relates multiple traits to predatory behaviour) than Iran does. Together with the outliers in Figure 1f (Costa Rican ants with either very small or very large mandibles), this result suggests that whilst similar on average, Costa Rica does have a greater diversity of ants along the specialistgeneralist gradient.

The Iranian fauna occupies only a narrow portion of trait space on axis 2. This suggests a trend of predatory generalism among desert species, with no investment in mandibular specialism in either direction. This may be evolutionary bet-hedging in which traits which may reduce short-term success are favoured in order to minimise longterm risk. Because investment in specialisation may not pay off in a harsh, unpredictable environment (VENABLE 2007), there is a smaller window of traits in desert ants. This generalism may also include scavenging behaviours exhibited by some desert dwelling ants (WHITFORD & al. 1980). If food sources are variable and random in nature it may be more advantageous to have less extreme foraging specialisation in terms of mandible size, meaning smaller food substrates are likely to be sufficient, and larger substrates are less likely to be too big to carry. The harsh, limited and unpredictable environment in the desert is likely to favour generalism over specialism in terms of foraging (KASSEN 2002). Iranian species cover most of the gradient in habitat openness in Figure 3, although the spread of species is skewed towards larger-bodied portion of the axis, with all smaller-bodied individuals comprised of Costa Rican species.

Morphological differences were not seen across the two biomes in all traits. Relative clypeus length has been proposed to relate to reliance on liquid food (SILVA & BRANDAO 2010). From the results, there was no difference in clypeal size between the two biomes (Tab. 1). While low levels of specialisation may be expected in the dry desert biome, some level of specialisation may be expected from the rainforest species. It may be that where rainforest species do utilise liquid resources, specialisation is unnecessary as resources are plentiful or easy to access. Similarly, there was no difference in measurements of relative pronotum width between the two biomes, meaning that body width changes no more than body size across the two regions.

Overall, this study has documented the level of morphological trait overlap between two contrasting biomes; it is likely this is a result of adaptation to two contrasting environments. Our results confirm a greater range of phenotypes in rainforest habitats but suggest that certain traits may be similar even where environments are largely different. The use of the PCoA and functional trait based analyses allows us to see the important sources of variation in each community. We reiterate that the effect of the different biogeographical contexts of Iran and Costa Rica on the results presented here is unknown. We emphasise that this first look at biome-level differences is a valuable step toward understanding global variation in ant morphology. Future comparisons of similar biomes in different biogeographic areas and of different biomes within the same biogeographic area would go much further in allowing us to assess exactly how the environment filters and shapes the morphology of ant faunas.

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