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A comparative approach to understanding factors limiting abundance patterns and distributions in a fig tree- fig wasp mutualism

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Numerous factors affect distribution and abundance. Due to their intimate host association, pollinating fig wasps are expected to occupy the entire distribution of their host fig. An understanding of the physiology of fig wasp species and the factors affecting adult survival are important for understanding and explaining wasp abundance and distribution patterns. We examine distribution and abundance patterns of a fig tree-fig wasp system in which the non-pollinator (*Ceratosolen galili*) has been reported to be rarer than the pollinator (*Ceratosolen arabicus*) in drier areas due to lower desiccation tolerance. Both species are closely related and utilise the same oviposition sites on *Ficus sycomorus*. We used ecological niche modelling to determine whether *C. galili* covers the range of *F. sycomorus* and whether it is consistently rarer than *C. arabicus*. We examined emergence times, critical thermal limits, desiccation and starvation tolerances of the wasps. The suggested rarity of *C. galili* relates to lower abundance in certain months and at certain localities but a similar number of occurrence records within the distributional range (except in arid regions, e.g. Namibia). In contrast to *C. galili*, *C. arabicus* has a wider thermal range, lives longer under hydrating and dehydrating conditions and is a nocturnal flier. The synergistic effect of physiological tolerances and flight time differences exacerbate the less favourable conditions available to *C. galili* during flight and location of receptive figs. These factors enable *C. arabicus* to survive for longer than *C. galili*, meaning that they are more likely to disperse to trees that are flowering further away in space and time. Combining correlative and mechanistic approaches has aided us in understanding the ecological niches of these species.

Physiology, species abundance patterns and distribution

A complex and varied set of factors may determine the range limits of a species, and the abundance structure across its range. Fine-scale changes in the distribution of suitable microclimates may lead to changes in population size and colonization rate and to changes in distribution (Wilson et al. 2009). The physiological tolerances of species may be one driver of these changes where, for example, individuals of a species are completely excluded from habitats experiencing climatic conditions outside of their physiological tolerances. Such an effect of humidity on abundance and distribution limits has been recorded in tsetse flies (Rogers and Randolph 1986), while in another study, the wider physiological tolerances of some crustaceans allowed them to occupy a wider geographical range (Gaston and Spicer 2001). In another example, on sub-Antarctic Marion Island, temperature and desiccation are known to exclude slugs from particular habitats (Lee et al. 2009).

Factors limiting distribution and abundance may vary within and over seasons (wet vs. dry and cold vs. hot, Wilson et al. 2009) and thus only operate at certain spatial and temporal scales (Kjellberg et al. 1988, Holt et al. 2005, Nielsen et al. 2005, Gaston and Fuller 2009), for example, when environmental conditions are less favourable. Therefore, we need to consider how these factors affect distribution and abundance patterns not only pooled over time but also within seasons.

Despite the value that physiological data may contribute to distribution modelling, these data are seldom available and consequently are rarely incorporated into mechanistic distribution modelling (Kearney and Porter 2009, Soberón 2009).

Distribution modelling and abundance patterns of fig tree- fig wasp mutualisms

The fascinating association between the organisms involved in fig tree- fig wasp mutualisms has become a model system for investigating numerous evolutionary and ecological questions (Janzen 1979, Wiebes 1979, Wang et al. 2008). In these mutualisms, the tree depends on the wasp for the dispersal of pollen and for seed set, while the wasp depends on the tree for offspring development (Wang et al. 2008).

In contrast to externally feeding insects, fig wasp pollinators are expected to occupy most of the geographical ranges of their hosts because of their intimate host associations (Wiebes 1979, Weiblen 2002). A shift in the host's distribution must entail a concomitant shift in the distribution of the pollinator for the mutualism to persist and *vice-versa*. If the host plant becomes extinct, the wasp will also become extinct unless it is able to shift to a new host plant. Furthermore, the environmental factors structuring tree populations may differ from those driving wasp populations. This can result in a mismatch of species fundamental niches, which may lead to a breakdown in the mutualism, especially under a rapidly changing climate (Hegland et al. 2009). Therefore, to understand which factors may be most important in determining fig tree-wasp distribution and abundance patterns requires investigating patterns of both organisms involved in the mutualism.

Soberón (2007, 2009) suggests combining data on abiotic factors, biotic factors and species dispersal as a more comprehensive and mechanistic approach to species distribution modelling (see also Pulliam 2000). We believe that this approach is useful to apply to both distribution modelling and species abundance patterns. For fig wasps, these factors include spatial and temporal variation in environmental conditions that affect host plant distribution and abundance. For example, tree water availability and climatic conditions may affect both the distribution and abundance of the fig tree host and its fruiting cycle, which in turn affect the wasps that utilise the fruit (Kjellberg and Valdeyron 1990).

The ability of the adult wasps to survive from emergence until finding and entering a receptive fig in which to oviposit is another essential factor affecting wasp abundance and distributions. The fig wasp does not need to be present throughout the year for pollination of distant trees to persist. The tree will receive pollen as long as some trees (within the dispersal distance of the wasps) contain wasps and these wasps are able to survive until either locating a distant tree or until the tree becomes receptive (Kjellberg et al. 1988). Indeed, Kjellberg and Valdeyron (1990) suggest that the pollinator of *Ficus sycomorus* L. may not be present in locations where the trees have been planted as it is unable to survive these conditions, while the pollinator for *F. carica* L. may be temperature limited. Also, range expansion of *Ficus* spp. and persistence of their pollinators may be limited by wasp developmental time and fig flowering phenology under cooler climatic conditions (Kjellberg and Valdeyron 1990, Bronstein and Patel 1992).

As fig wasps do not feed as adults (Compton et al. 1994), starvation tolerance may be particularly important under dry (winter) conditions where individuals would require more searching time to find suitable oviposition sites. Under drier conditions, fewer seedlings may grow and establish and fig trees may be less abundant, and thus spaced further apart (see Wharton et al. 1980, Greeff unpublished data). Also, fruiting cycles may be severely affected by drier conditions (less frequent fruiting and smaller crop sizes, Greeff and Compton unpublished data). A species that has a greater starvation tolerance would be able to search for longer (i.e. able to resist starvation for longer) and would be more likely to find a suitable host (see also Kjellberg et al. 1988).

In addition, the wasps may be more susceptible to unfavourable environmental conditions at certain times of the day. For example, a temporal structuring of emergence times may allow nocturnally emerging individuals to survive unfavourable seasons while diurnally emerging individuals succumb to high temperature and low humidity. Thus examining

behavioural differences between species may yield some insight into differences in distribution and abundance patterns.

Therefore, the factors affecting adult survivorship and oviposition while dispersing from the natal tree include dispersal distance and distance to nearest fruiting tree, climatic conditions, physiological (e.g. thermal, desiccation and starvation) tolerances and behavioural constraints (e.g. the time of day of emergence from the fig syconium) (Kjellberg et al. 1988, Compton et al. 1991, 1994).

Adopting a comparative approach to understanding *Ficus sycomorus-Ceratosolen* distribution and abundance patterns

Ficus sycomorus L. is distributed across a large part of Africa, Madagascar and the Arabian Peninsula (Kerdelhue et al. 1999). It is believed to have originated in Madagascar with the tree and its pollinator, *Ceratosolen arabicus* Mayr, secondarily colonising the African continent (Kerdelhue et al. 1999). *Ceratosolen galili* Wiebes also utilises the figs of *F. sycomorus* but rarely pollinates any of the flowers (Compton et al. 1991). *C. galili* is believed to have been the pollinator of another, currently extinct, African *Ficus* species but was able to survive through a horizontal transfer to a new host plant species, namely *F. sycomorus* (Kerdelhue et al. 1999). Both species are likely to compete for oviposition sites inside figs and also to compete for figs. After entry and oviposition by a female/s, the fig is no longer attractive to the wasps, preventing further wasp individuals from entering (Compton et al. 1991) thereby reducing founding possibilities for other females, including heterospecifics. This may lead to patterns where higher abundance of one species will result in lower abundance of the other.

Several authors have noted differences in the distribution and abundance patterns of these two wasp species. Specifically, *C. galili* has been reported to be rare in drier areas

(Wharton et al. 1980, Compton et al. 1991). Although rarity may be defined in terms of a species abundance or occupancy, with rare species occurring at low abundance and/or occupying few localities (Gaston 1994), the authors do not qualify what they mean by rarity nor can we determine this from their publications. However, *C. galili* may well occupy fewer sites and occur at lower abundances in drier areas. Galil and Eisikowitch (1968) state that *C. galili* appeared to be more abundant at the coastal, and thus more humid Kenyan coastal town of Mombasa, than in the interior of the African continent (This is the only locality where the authors recorded the species in Kenya over a period of four months, D. Eisikowitch pers. comm.). Also, five trees that were sampled along the Ugab River, Namibia in 2003 contained only *C. arabicus* and no *C. galili* (S. G. Compton pers. comm.). Therefore, questions arise as to what form of rarity (distribution, abundance or both) is present and why we find differences in the wasp abundance/distribution patterns, even though they are utilising the same tree host.

C. galili is suspected to be a day-flier while *C. arabicus* is collected abundantly at nocturnal light traps (Compton et al. 1991). This means that due to its flight time, *C. galili* would be exposed to higher temperatures and lower humidity than *C. arabicus* in all areas where both wasp species occur. *C. galili* may thus be overcome by either extreme temperatures or desiccation, concomitantly lowering abundance in sub-optimal localities and ‘contracting’ its distribution. In addition, *C. galili* has been suggested to be less desiccation resistant (Wharton et al. 1980, Compton et al. 1991) and may also be less temperature tolerant than *C. arabicus*, and its numbers may be severely reduced due to physiological intolerance, in addition to, its diurnal flight time. The synergistic effect of the behavioural and physiological differences is likely to magnify differences in distribution and abundance of the species. However, these effects may only play out at certain times of the year when environmental conditions are less favourable. This would have the effect of reducing the number of ‘sink’ populations and temporarily lowering abundance of ‘source’ populations and contracting

distribution. Temporal fluctuations will be unnoticeable when plotting distribution from historical records collected over multiple seasons and years but the ‘signal’ in abundance data should remain when examining such data across seasons or within seasons and across localities.

The aims of our study were to first, determine whether *Ceratosolen galili* covers the entire distribution range of *Ficus sycomorus* and second, to ascertain whether *C. galili* is consistently rarer than *Ceratosolen arabicus* at specific locations within and across seasons. Our third aim was to investigate the potential behavioural and physiological underpinning of differences in distribution and/or abundance of the wasp species. The emergence times of the species were used to estimate the time of day that these insects fly and reflect a potential behavioural driver of different wasp distributions and abundance patterns. To assess potential differences in physiological tolerances, we determined the upper and lower critical thermal limits, desiccation and starvation tolerances (at a constant temperature) for both species.

Materials and Methods

Distribution modelling

Data

The natural distribution region of *Ficus sycomorus* L. extends from Sudan and Ethiopia in the north of Africa to South Africa and Madagascar (Galil and Eisikowitch 1968, Kerdelhue et al. 1999). *F. sycomorus* in the Kruger National Park, South Africa is close to the southern

distribution limit for the species in Africa. Both *Ceratosolen arabicus* and *C. galili* appear to have large geographical ranges and are dependent on *F. sycomorus* for their reproduction. Data on the distribution of *Ficus sycomorus* (subspecies *sycomorus* and *gnaphalocarpa*) and its pollinator (*C. arabicus*) and cuckoo wasp (*C. galili*) were collated from multiple sources, including the PRECIS database of the South African National Biodiversity Institute and original records collected by J. Galil and D. Eisikowitch and other researchers (see acknowledgements). When records did not include coordinates for localities, we extracted these from electronic gazetteers, like GeoNet (<http://earth-info.nga.mil/gns/html/index.html>). To avoid pseudo-replication we removed all duplicates. For each species we used only one record per 2.5 minute grid cell as defined by the environmental predictor variables (see below). After removing duplicate records in grid cells, 176 records were available for *Ficus sycomorus*, 71 records for *C. arabicus* and 73 records for *C. galili*. *F. sycomorus*, *C. arabicus* and *C. galili* are known to occur in West Africa. This region was not included in our analysis as we were unable to obtain distribution data for these species from this region.

Raster geospatial data sets, used to characterise environments across the map region, consisted of ‘bioclimatic’ variables interpolated at 2.5 minutes spatial resolution (Hijmans et al. 2005). The variables used included annual mean temperature, mean diurnal range, maximum temperature of warmest month, minimum temperature of coldest month, annual precipitation, and precipitation of the wettest and driest months. These variables were chosen to summarize temperature and moisture trends that were considered to be most relevant for limiting the distributions of the tree and the wasps. The same selection of variables has been used successfully to predict the distribution of other small arthropods (De Meyer et al. 2009).

Ecological niche modelling

Our approach is based on the idea of modelling species' ecological niches, which are considered to constitute long-term stable constraints on species' potential geographic distributions (Martínez-Meyer et al. 2004, Peterson et al. 1999, Raxworthy et al. 2003, Wiens and Graham 2005). Ecological niches are defined as the set of conditions under which a species is able to maintain populations without immigration (Soberón 2007).

We used a correlative ecological niche modelling (ENM) technique (Maxent, Phillips et al. 2006) to estimate the potential distribution of all three species. Maxent estimates the ecological niche of a species by determining the distribution of maximum entropy, subject to the constraint that the expected value of each environmental variable (or functions of these) under this estimated distribution matches its empirical average (Phillips et al. 2006). Maxent makes use of presence records and a set of background values drawn from the entire study region. We used default parameters in Maxent (version 3.1.0) to produce models: feature selection automatic, regularization multiplier at unity, maximum iterations 500, convergence threshold 10^{-5} , and random test percentage at zero. We produced maps using the logistic output format.

Model evaluation

We evaluated model prediction success by assessing the accuracy with which the models were able to predict the occurrence records that were used to train the models for each species. We also randomly split the dataset into 70% training records (for model calibration) and 30% evaluation records (as an independent testing set). We repeated this five times. The Area Under Curve of a Receiver Operating Characteristic (ROC) curve was used to evaluate overall model performance (Fielding and Bell 1997). As we did not have any reliable absence data, we used the AUC values from ROC curves as calculated by Maxent because they do not

require absence data (Phillips et al. 2006). Maxent uses the fraction of the total study area predicted present on the y-axis of the ROC curve, instead of the commission error rate (the calculation of which requires absence data) in order to calculate an AUC value (Phillips et al. 2006).

Abundance patterns

Hengeveld and Haeck (1982) state that abundance or frequency of occurrence may be used to estimate 'intensity' or expected number of individuals per unit area. A dataset (independent from that used to determine the abundance patterns below) collected between October 2004 and February 2006 from 73 Kruger National Park (KNP), South Africa trees revealed that proportional occupancy (number of occupied figs per tree/ total number of figs sampled) and total or mean abundance per tree were highly correlated ($r = 0.89$ and 0.95 respectively, $p < 0.001$). We therefore used proportional occupancy as the abundance measure across all localities. Data on *C. arabicus* and *C. galili* occupancy of figs on individual trees at different locations were obtained from published surveys: Namibia, collected in December 1978 (Wharton et al. 1980) and KwaZulu-Natal, South Africa, collected in December 1988 (Compton et al. 1991), and unpublished data collected in May and December 2005 in KNP. These data are thus from three regions within the distribution of the tree and wasps. For each tree, 25-50 figs were sampled to estimate proportional occupancy per tree (except for Namibia where 300 figs from a single tree were sampled).

We first examined the relationship between abundance (proportional occupancy) across the three localities for the two species, standardised for season by only using collections for summer (December) using simple regression in Statistica v5.5 (Zar 1996). We then examined

the relationship within a single locality (KNP) between seasons (summer, $n = 19$ trees and winter, $n = 24$ trees). We performed separate regressions for each season and compared the slopes of the regressions using an F-test (Zar 1996). As the slopes did not differ significantly ($F_{1,39} = 0.21$, $p = ns$), we then proceeded to test if the intercepts differed between seasons using Analysis of Covariance (Zar 1996).

Behavioural trials: Emergence times

To record emergence times, mature figs were collected from Olifants and Skukuza Rest Camps, Kruger National Park, South Africa in February 2004 and June 2008 respectively. Figs were housed individually in 100ml plastic jars covered with organza. The figs were observed every hour and the time of wasp emergence, species and number of individuals were recorded for each fig. A single species emerged from 88/140 and 35/83 figs over the two collections. These single species figs were used to determine emergence times to prevent bias in the estimates resulting from multiple species emerging from the figs. Because emergence time data are grouped, it is recommended that contingency tables be used rather than circular statistics (Zar 1996). Chi-square tests were used to test if emergence times are uniform and a contingency table was used to compare the eclosure pattern of the two species.

Physiological trials

Differences in thermal and desiccation tolerances between the species may affect distribution and abundance. Ascertaining if the species have different critical thermal limits and desiccation limits may contribute to our understanding of differences in distribution and abundance patterns. Although clines in thermal and desiccation tolerance have been found for some

species and should be tested across a species' range, this would involve an exhaustive study of the thermal biology of these species across Africa (which would yield insights into spatial variation in these parameters). However, because fig wasps are able to disperse over vast distances (Jansen van Vuuren et al. 2006 found no genetic differentiation in two pollinating wasp populations that are 500km apart), local adaptation and the formation of thermal and desiccation clines, may not be possible for fig wasps and are thus unlikely to influence our results. We tested the thermal and desiccation tolerances of these species in an area where both wasp species occur and that is close to the southern range limits of the host plant.

All experiments were performed on one population that was sampled in Skukuza Rest Camp, Kruger National Park (KNP), South Africa from two *F. sycomorus* trees. Fresh, mature figs (n =26), which were close to releasing live wasps, were collected and immediately taken to the field laboratory. Figs were sliced in half to stimulate the female wasps to emerge. Emerging wasps were immediately used in the experiments (below). Sampling commenced in February 2008 during the traditional peak of summer temperatures and after good rainfall (Skukuza Weather Station, SANParks). Winter in KNP is defined predominantly by lower rainfall, not decreased temperature, compared to summer and occurs from May to August (H. Sithole pers. comm.). Experiments were conducted on females of the species as the males mate inside the controlled fig environment and do not disperse.

Determining critical thermal minima and maxima

The critical thermal minimum, identified as the onset of (CTMin_o) and recovery from (CTMin_r) chill coma, and critical thermal maximum, identified as the onset of spasms (CTMax), was determined for *C. arabicus* and *C. galili* females. Ten specimens were collected immediately on emergence and placed individually into 1.5ml ependorfs with transparent lids. The

ependorfs were submerged in a Labotec water bath connected to a Julabo P temperature controller. Because of the small body size of these wasps, the body temperatures were assumed to be equivalent to ambient temperature (Huey et al. 1992). The bath's temperature was left to stabilize for 15 minutes at 14°C after which it was lowered at approximately 0.25°C.min⁻¹ until the onset of chill coma (CTMin_o) was recorded in all animals. The temperature at which a particular specimen was unable to right itself after turning it on its back was noted as the CTMin_o for that individual (Klok and Chown 1997). A fine paintbrush, held at bath temperature, was used to manipulate the animals. The bath temperature was allowed to drop 0.5°C lower than the last CTMin_o value measured. The animals were held there for 5 minutes to allow for equilibration and the temperature was then increased at the same rate. The temperature at which an individual regains complete motoric functioning (regained the ability to right itself) was noted as the CTMin_r for that individual. The wasps were discarded and the water bath was left to increase its temperature to 27°C, where it was allowed to stabilise. Ten fresh specimens were submerged in separate ependorfs and the bath's temperature was increased. The temperature observed at the onset of muscular spasms in an animal was noted as the CTMax for that particular animal (Klok and Chown 1997). The procedure was repeated three times for each species for CTMin and CTMax to give a total n = 30 per species. The critical thermal range (CTRange) for each species was calculated as the difference between the average CTMax and CTMin_r values.

Generalised linear models with normal error structure and identity link functions were used to evaluate the effects of species, replicates and their interactions, on critical thermal measurements (STATISTICA version 5.5, McCullagh and Nelder 1989). The best subsets likelihood ratio approach was used to determine the best-fit model with fewest terms (McCullagh and Nelder 1989, Collet 1991, Dobson 2002). Goodness of fit was measured using the deviance statistic and the percentage deviance explained (similar to R²) for the best fitting

model was calculated. The change in deviance for single variables was used to estimate the contribution of individual variables to the deviance explained by the final model (Collet 1991).

Determining desiccation and starvation resistance

Thirty freshly emerged female wasps were placed individually into 1.5ml ependorfs with both ends covered with organza. Each ependorf was placed into its own miniature desiccation chamber containing silica gel crystals at ambient temperature ($27 \pm 1.5^\circ\text{C}$) and initially at ambient humidity. Adult female wasps were monitored every hour until all wasps were dead. At the same time, 30 adult females were placed in ependorfs in control chambers at the same ambient temperatures, but with distilled water instead of silica gel crystals, in the chambers. From this we were able to establish how rapidly death occurred under the desiccation or starvation treatment. The experiment was repeated for the second species.

To determine if the two species exhibited differences in terms of survival times under different environmental conditions, survival analysis was executed, using the survival library, in R 2.7.1 (R Development Core Team; <http://www.R-project.org>). The Cox proportional-hazards regression model was fitted with the `coxph` function (Dalgaard 2004). Data were not censored, except for *C. galili* under hydration (but this was only after 50% of the individuals in the treatment had died). Proportionality of hazards was confirmed with the `cox.zph` function.

Results

Distributions

The potential distribution of *Ficus sycomorus* is extensive (Fig. 1). Regions of high suitability predicted by the ecological niche model include the Arabian peninsula, Ethiopia, east Africa and most of southern Africa (Fig. 1). Parts of the north African coast and parts of Madagascar are also predicted to be highly suitable. The distribution records of *F. sycomorus* coincide reasonably well with regions of high predicted suitability (>0.15), except along the southern and eastern coasts of South Africa which have a high suitability but no distribution records. The AUC value for the model evaluated using all of the training records was 0.963 and the average AUC value taken from five models evaluated using 30% randomly selected evaluation records was 0.918. Many more distribution records of *F. sycomorus* are available in southern Africa (especially South Africa, Namibia and Botswana) than in east and north Africa, where the species is known to occur. The predicted distributions of the two wasp species are quite similar and show similar potential distributions to that of *Ficus sycomorus* (Fig. 1). *C. galili* shows a smaller region of high suitability (>0.15) in the drier south western parts of southern Africa than *C. arabicus*. The sizes of the distribution ranges of the two species appear to be similar, although the areas of high suitability (>0.15) for *C. arabicus* are about 30% larger than for *C. galili* (Fig. 1). The model for *C. arabicus* obtained an AUC value of 0.957 when using all training records and a mean value of 0.935 when evaluated with the 30% evaluation set. The model for *C. galili* obtained an AUC value of 0.970 when using all training records and a mean value of 0.949 when evaluated with the 30% evaluation set. Swets (1988) suggested that models with AUC values that are greater than 0.9 are considered to be excellent. The models for all three species can be considered to show excellent model performance using the AUC statistic. Although model performance was similar across species, on average models for *C. galili* performed best, followed by that for *C. arabicus* and then *F. sycomorus*.

Rainfall of the driest month and minimum temperature of the coldest month, followed by annual precipitation, were shown to be important environmental variables predicting the wasp distributions but were not consistently important for tree distribution (Table 1). The sets of distribution records used to calibrate the models for the two wasp species show considerable overlap in climate space (Fig. 2).

Comparing abundance patterns

As *C. arabicus* occupancy (and thus abundance) of figs increased, *C. galili* occupancy decreased across localities within the summer season ($F_{1,29} = 83.08$, $R^2 = 0.73$, $p < 0.001$, Fig. 3a). *C. arabicus* was more abundant in Kruger National Park (Mean \pm SE: *C. arabicus*: 0.78 ± 0.04 ; *C. galili* 0.27 ± 0.04), while *C. galili* was more abundant in KwaZulu-Natal province of South Africa (Mean \pm SE: *C. arabicus* 0.30 ± 0.06 ; *C. galili*: 0.81 ± 0.04) in the summer. *C. galili* was absent from the tree sampled in Namibia in 1978 (and has been shown to be absent during a subsequent survey of five trees in 2003, S. Compton pers. comm.).

Across seasons, a similar pattern is visible, with a decrease in *C. galili* occupancy as *C. arabicus* occupancy increases (Table 2, Fig. 3b). Furthermore, even when *C. arabicus* occupancy of figs is low, *C. galili* occupancy is at lower levels in almost every case. Although *C. arabicus* occupancy (and thus abundance) did not vary significantly between winter and summer seasons (0.78 ± 0.03 and 0.78 ± 0.04 respectively), *C. galili* occupancy is lower in the drier winter months (Table 2, Fig. 3b).

Comparing behaviour: emergence times

The mean emergence time of *C. arabicus* females was at 20h20 and it was significantly different from a uniform emergence pattern ($\chi^2 = 55.5217$, $df = 5$, $p < 0.001$, times were collapsed to 6 periods: 2am-6am, 6am-10am, 10am-2pm, 2pm-6pm, 6pm-10pm, 10pm-2am, Fig. 4). By contrast, *C. galili*'s mean emergence time was almost exactly 12 hours later at 08h55 and its emergence was also significantly different from a uniform distribution ($\chi^2 = 22.5714$, $df = 5$, $p < 0.001$, times were collapsed to 6 periods as above, Fig. 4). The emergence times of *C. arabicus* and *C. galili* differ significantly from one another ($\chi^2 = 34.4778$, $df = 5$, $P < 0.001$).

Comparing physiology: temperature, desiccation and starvation

No significant differences were found between replicates (Wald = 4.32, $p = 0.12$) or species (Wald = 2.50, $p = 0.11$) for the onset of chill coma (Deviance= 38.65, deviance/df = 0.73, Fig. 5). The onset of chill coma was similar for both species (Mean \pm S.E. *C. arabicus* $5.76 \pm 0.14^\circ\text{C}$, *C. galili* $5.43 \pm 0.18^\circ\text{C}$). However, recovery from chill coma (CT_{min_r}) was significantly slower and at a higher temperature for *C. galili* ($13.90 \pm 0.21^\circ\text{C}$) than for *C. arabicus* ($12.52 \pm 0.15^\circ\text{C}$) (Table 3, Fig. 5). Although significant differences between replicates were apparent for CT_{Min_r}, the species effect was greater. The critical thermal maximum temperature for *C. galili* ($37.12 \pm 0.42^\circ\text{C}$) was significantly lower than for *C. arabicus* ($40.07 \pm 0.28^\circ\text{C}$) (Table 3, Fig. 5). The critical thermal ranges differed by approximately 4°C (27.55°C and 23.22°C for *C. arabicus* and *C. galili* respectively). Therefore, *C. galili* is more susceptible to extreme temperatures than *C. arabicus*.

C. galili females always died significantly earlier than *C. arabicus* females in hydrating and dehydrating environments and all individuals in the dehydrating environment died significantly faster than those in the hydrating environment (Fig. 6, Table 4). The effect of treatment (namely higher or lower relative humidity) was much greater than that of the differences between the species, although both effects were significant (Table 4). These results show that desiccation and starvation affect the survival of both species, more so for *C. galili* than *C. arabicus*.

Discussion

Distribution

The potential distributions for the two wasp species appear to be very similar, despite having different physiological tolerances. Since the approach taken to define the potential distributions of these species was correlative, the quality of the datasets of distribution records used to develop the models should be considered. The distribution records were not collected by means of a systematic survey in which the presence or absence of each species was recorded at a set of localities. This approach is preferable for developing correlative models but is rarely practical (Funk and Richardson 2002), especially when the ranges are very large as is the case here. The only data available to us consisted of presence records that were collected on an *ad hoc* basis, which have a number of associated limitations (Funk and Richardson 2002). The distribution records for these species may not have adequately sampled the ranges of these species and may have been insufficient to show real differences in potential distributions

between these species. Many of the grid cells making up this dataset were occupied by both species (61 grid cells) and only a limited number were occupied by one species and not the other (12 where only *C. arabicus* was present and 10 where only *C. galili* was present). For this reason the species appear to occupy very similar climate spaces (Fig. 2). It is also clear from the spatial distribution of the records that more sampling effort was invested in southern Africa than elsewhere in the map region. In most cases distribution records are found in regions of high suitability and model performance was considered to be excellent. However, for *C. arabicus* three distribution records occur in Namibia even though predicted climatic suitability for this region is low. This may be because the species is able to occupy microclimates within this region that are not well described by the relatively coarse-grained (2.5 min) predictor variables (De Meyer et al. 2009). For example, *F. sycomorus* trees often grow on river banks in arid areas and have access to water from the river which did not necessarily fall in that area. The amount of water available to the trees in these grid cells will not necessarily be well described by the annual precipitation of these grid cells. This could result in lower than expected model suitability in arid regions.

C. galili has yet to be recorded in Namibia and thus far appears not to be present in that country despite a number of collections in the region (1978, 2003, 2008). Namibia lies on the drier western side of southern Africa and represents climatic conditions that are too hot and dry in the summer and too cold in the winter and generally have low predicted suitability for, not only, *C. arabicus* and *C. galili* but also for their tree host. Furthermore, trees are spaced substantially further apart along the Kuiseb River, Namibia (density of 57 trees over 120 km) than along the rivers in KNP, South Africa (Wharton et al. 1980, pers. obs.). Specifically, tree density along perennial rivers is higher (>59 trees in 2.2 km along one side of the river) than along ephemeral rivers in KNP (56 trees over 11 km) (pers. obs.). The higher the tree density, the nearer a receptive fig tree is for newly emerged female wasps. As the distance between

trees increases, wasps need to search for longer, and thus need to survive longer under ambient conditions, before they are able to find a receptive fig tree. Namibian ambient conditions are clearly less favourable than, for example, KNP ambient conditions for these wasps.

Comparing behaviour and physiology and their potential effects on distribution and abundance patterns

Although the distribution data suggest that both species occupy the same climate space, significant physiological and behavioural differences were apparent between the wasp species. Dispersal ability is (most likely) similar between the two species: as both species have a small body size, they are able to disperse large distances via wind transport (Nason et al. 1996, Jansen van Vuuren et al. 2006, Harrison and Rasplus 2006). Dispersal ability would thus be expected to affect both species' distributions in a similar manner. But longevity differs between the species, especially under dehydrating conditions. Indeed, wasps that are able to survive for longer are more likely to be able to disperse to trees that are flowering further away in space and time (Janzen 1979, Kjellberg et al. 1988). We show that in dry *and* in humid conditions, *C. arabicus* is able to live longer than *C. galili*. Fig wasps are known to be short-lived (Kjellberg et al. 1988), however, under optimal conditions (high relative humidity and 27°C) we found that *C. arabicus* was able to survive for up to five days. This survival time is significantly longer than *C. galili*, and would lengthen the time that *C. arabicus* individuals have to find receptive fig trees.

In addition to longevity differences between the species, *C. arabicus* is able to survive a wider thermal range than *C. galili*, enabling it to survive a wider variety of microclimates. Part of the answer to these differences in physiological tolerances lies in *C. galili* being smaller in

size than *C. arabicus* (see Compton et al. 1991) as size is known to affect an insect's ability to resist desiccation (Chown and Gaston 1999). Indeed, *C. galili* is less desiccation tolerant than *C. arabicus*. As suggested by Compton et al. (1991), we have shown that *C. arabicus* is a nocturnal flier. This would mean that *C. galili*'s diurnal flight time would increase the exposure of individuals of this species to high temperatures and dehydrating conditions. However, the synergistic effect of differences in physiological tolerances and flight times, would further exacerbate the less favourable conditions available for adult *C. galili* during flight and location of receptive figs. Such effects are likely to lower the abundance of *C. galili* relative to *C. arabicus* when environmental conditions are less favourable.

Other factors limiting distribution and abundance patterns

Although the environmental factors structuring tree and wasp distributions were broadly similar, not all environmental variables contributed to the same extent, nor were all the same variables of equal importance to all partners involved in the mutualism. For example, maximum temperature of the driest month was more important in contributing to *F. sycomorus* distribution than to the wasp distributions. Also, precipitation in the driest month and annual precipitation largely contribute to wasp distribution but not to the tree. This is because the trees are less likely to be water restricted than the wasps after they have grown and established along the river bank because they have access to water from the river and their deep root system enables them to access below ground water. The fact that maximum temperature was not important for wasp distributions may be explained by the fact that trees transpire actively to keep fruits cooler (Patiño et al. 1994). These differences in contributing variables may effect a mismatch in the host and wasps' species fundamental niches under an increasing temperature

climate scenario (Hegland et al. 2009). It is presently uncertain how generalisable these results are across mutualisms (Hegland et al. 2009). However, our behavioural and physiological results show that the direct effect of temperature on *C. galili* may be greater than the effect on the pollinator, *C. arabicus*.

Species interactions may also have affected the predicted distributions over and above macro-environmental, physiological and behavioural factors (Gaston 2003, Gaston and Fuller 2009, Soberón 2009). Both species utilise the same structural component of the plant (fig flowers) on the same tree species. The first species to arrive at the receptive figs, will enter the figs immediately. After entry and oviposition by a wasp/s, the fig is no longer attractive to the wasps, preventing further wasp individuals from entering (Compton et al. 1991). The wasp species with highest abundance at that time will ‘close’ the figs to entry by the other species. Indeed, when pollinator (*C. arabicus*) abundance is high, *C. galili* abundance is lowered across localities and *vice-versa*.

This clear relationship between *C. arabicus* and *C. galili* abundance (measured as occupancy of figs per tree sampled) may, in part, have contributed to the supposition of *C. galili*'s rarity. *C. galili* is rarer at localities where *C. arabicus* is abundant. The relationship between the abundance of the two wasp species holds across localities and seasons. Abundance is lower in the colder and drier months of the year and it is specifically *C. galili* abundance that is lower. These variations in abundance and distribution across seasons may explain the observation that *C. galili* is rarer than *C. arabicus* when sampling at a specific time. However, *C. galili* has been recorded on a similar number of trees as *C. arabicus* for the dataset at three localities (both species were collected from 30/32 trees) and the same abiotic conditions appear to limit their distributions. Also, when standardising for season, we can see that at some localities (e.g. KwaZulu-Natal) *C. galili* is more abundant in the summer than *C. arabicus*. Therefore, the suggested rarity of *C. galili* relates to lower abundance in certain months and at

certain localities but, in general, a similar number of occurrence records within the distributional range (except in arid regions, such as, Namibia). Thus rarity of *C. galili* vs. *C. arabicus* relates to abundance across their distributions and occupancy at certain, arid localities.

Accurate distribution and abundance data is severely lacking for most invertebrate species (Leather et al. 2008). As the collection of such data is time consuming and expensive when the spatial extent is large, adding new records is no simple task. Even for this study, where sampling occurred at numerous localities across years, data deficiencies may influence the distribution and abundance results. It is uncertain at present to what extent (number of localities) and how consistently (in terms of abundance) *C. galili* may be considered to be rare compared to *C. arabicus*.

Spatial and temporal variation in abundance exists. Because of the differences in wasp abundance on a tree, this would mean (i.t.o. metapopulation structure) that more immigrants and emigrants and more source and sink populations are present for *C. arabicus* than *C. galili*. *C. arabicus* should therefore be more common than *C. galili* if a positive abundance-occupancy relationship exists (Gaston et al. 2000). The relationship holds for both summer and winter seasons - at least for the Kruger National Park populations. It is not clear whether this relationship holds across localities.

To further our understanding of the distribution and abundance of these species we need to improve the correlative models (based on distribution records) and our mechanistic understanding by further exploring the physiological and behavioural interactions of these species. Both correlative and mechanistic approaches to understanding ecological niches are of great value (Kearney and Porter 2009).

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Figure Legends

Figure 1. Distribution predictions for a) *F. sycomorus* (showing countries and all distribution records), b) *C. arabicus* and c) *C. galili*. The darkest shades indicate regions of highest probability (potential environmental suitability). B – Botswana, E – Ethiopia, KNP – Kruger National Park, M – Madagascar, Mz – Mozambique, N – Namibia, SA – South Africa, Z – Zimbabwe.

Figure 2. The climate space occupied by *C. arabicus* (squares) and *C. galili* (solid) plotted on two axes from a principal components analysis performed on the matrix of predictor variable values associated with the distribution records. PC1 accounted for 43% of the variation. Minimum temperature of coldest month, precipitation of wettest month, annual precipitation and annual mean temperature had the highest loadings on PC1. PC2 accounted for 29% of the variation. Maximum temperature of warmest month, mean diurnal temperature range and annual mean temperature had the highest loadings on PC2.

Figure 3. Relationship between proportional occupancy of the two wasp species a) across three localities in Africa in summer (December) (KwaZulu-Natal, South Africa (KZN), Kruger National Park, South Africa (KNP) and Namibia) and b) across seasons (summer: circles and solid line, $F_{1,17} = 21.48$, $R^2 = 0.53$, $p < 0.001$; winter: squares and dashed line, $F_{1,22} = 8.60$, $R^2 = 0.25$, $p < 0.01$) for 19 and 24 trees, respectively, in KNP.

Figure 4. Emergence times (number of figs with emerging females) for *C. arabicus* (left) and *C. galili* (right) on a 24 hour clock.

Figure 5. Critical Thermal (CT) temperatures for *C. arabicus* and *C. galili* females. Open circles: CT maximum, open squares: onset of chill coma (CTMin₀), closed squares: recovery from chill coma. Bars represent ± 1 SE for each of 10 specimens. Temperatures differed significantly between species for recovery from chill coma and CT maximum. See text for details.

Figure 6. Estimated survival functions for both species and treatments. Point-wise 95% confidence intervals did not overlap and are not shown for clarity.

Table 1. Percentage contribution of the environmental variables to the distribution models that were calibrated and evaluated using all distribution data for each of the three species (calculated by Maxent v. 3.1.0).

Contribution of environmental variable	Species		
	<i>C. arabicus</i>	<i>C. galili</i>	<i>F. sycomorus</i>
Precipitation of Driest Month	25.9506	27.5822	8.5608
Min. Temperature of Coldest Month	25.6813	28.5948	21.2211
Annual Precipitation	16.5868	17.4984	5.972
Annual Mean Temperature	13.3216	8.2316	14.4055
Precipitation of Wettest Month	11.6833	14.8128	21.7783
Mean Diurnal Range [§]	5.0518	3.2721	2.435
Max. Temperature of Warmest Month	1.7245	0.008	25.6273

[§]Mean of monthly (maximum temperature - minimum temperature)

Table 2. Results of analysis of covariance for *C. galili* abundance between seasons, with *C. arabicus* abundance as covariate ($R^2 = 0.43$, $F_{2,40} = 16,59$, $p < 0.001$).

Covariate and factor	df	F	p <
<i>C. arabicus</i> abundance ^{\$}	1	26.73	0.001
Season	1	6.73	0.05
	<u>n (trees)</u>	<u><i>C. galili</i> abundance ± SE</u>	
Summer	19	0.27 ± 0.04	
Winter	24	0.16 ± 0.03	

^{\$} abundance measured as proportional occupancy, see text for details

Table 3. Best subset generalized linear models for recovery for chill coma (CTMin_r) and critical thermal maximum (CTMax) (°C) and the independent terms species and replicate. Only variables that were significant are shown. The estimate and the estimated percentage deviance explained (% Deviation) by the variables in the model are also provided.

Variable	df	Log likelihood	X^2	Estimate	% Deviation	p <
CTMin _r (% deviance explained=43.95, deviance/df=0.86, df=53)						
Species	1	-87.53	26.23	-0.69	33.6	0.001
Replicate	2	-79.04	9.24	0.54	10.6	0.01
CTMax						
Species	1	133.31	27.20	-2.95	37.43	0.001

Table 4. Results of Cox proportional hazards model, including the coefficients (\pm SE), exponentiated coefficients, z (ratio of regression coefficients to SE) and p-value. $R^2 = 0.843$, likelihood ratio test = 224 on 2 df, $p < 0.0001$.

Variable	Coef \pm SE	Exp (coef)	z	p <
Species	2.80 \pm 0.37	16.5	7.68	0.0001
Treatment	7.07 \pm 0.84	1175	8.45	0.0001

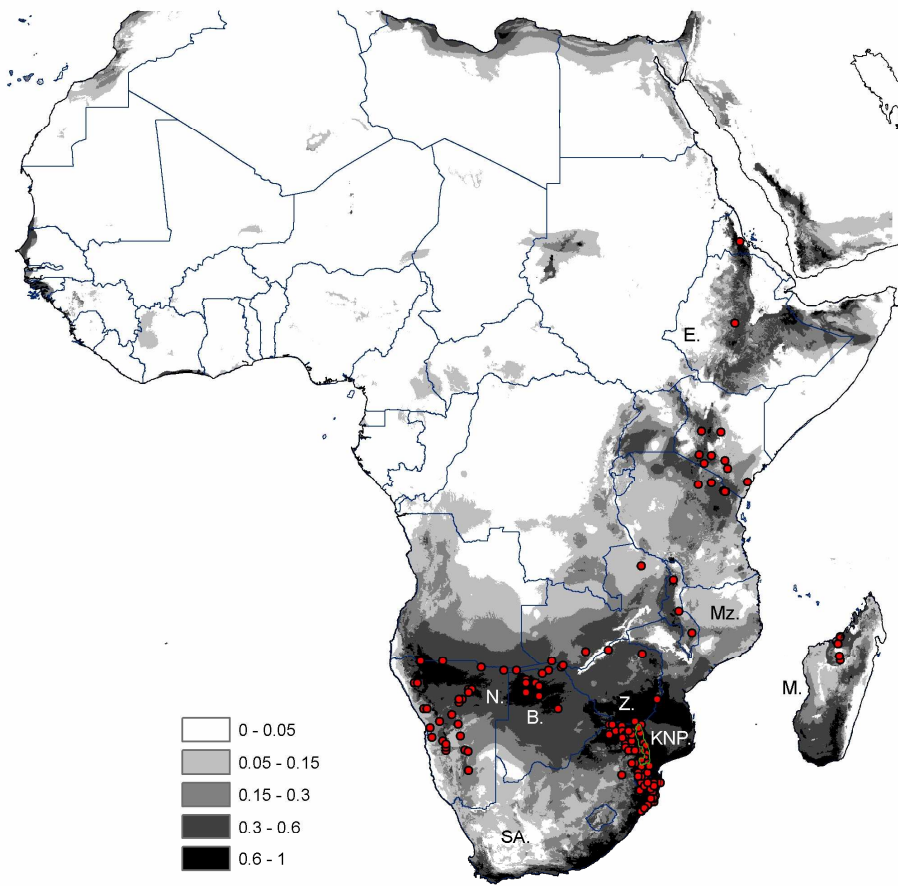


Figure 1a

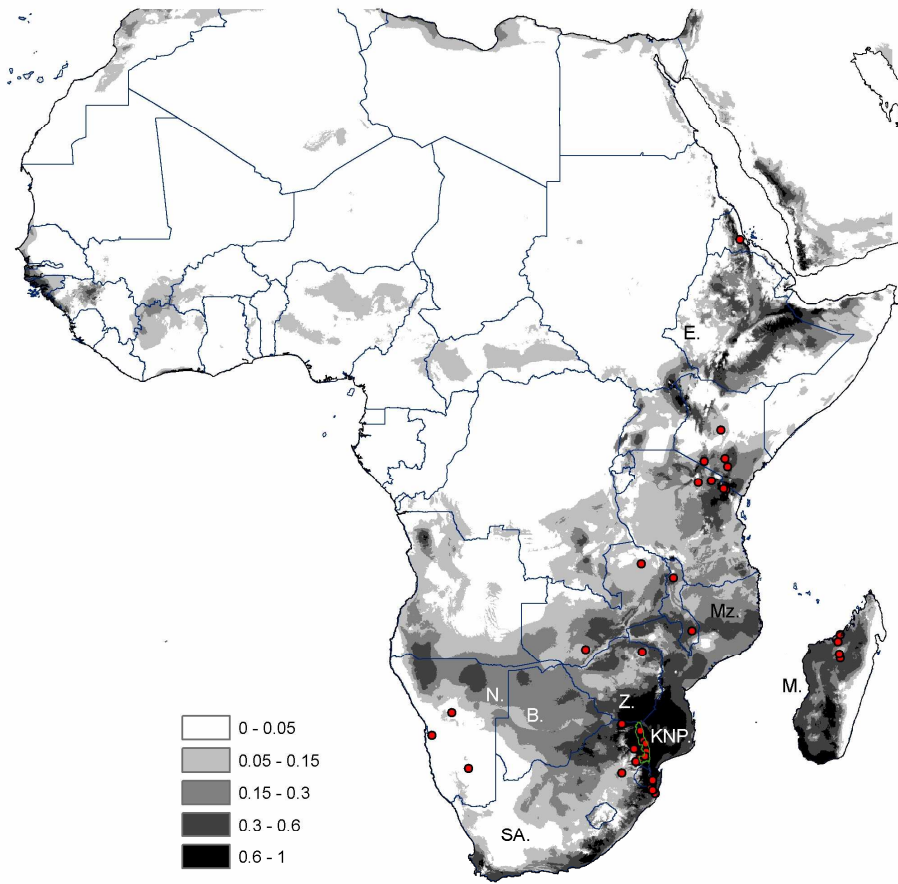


Figure 1b

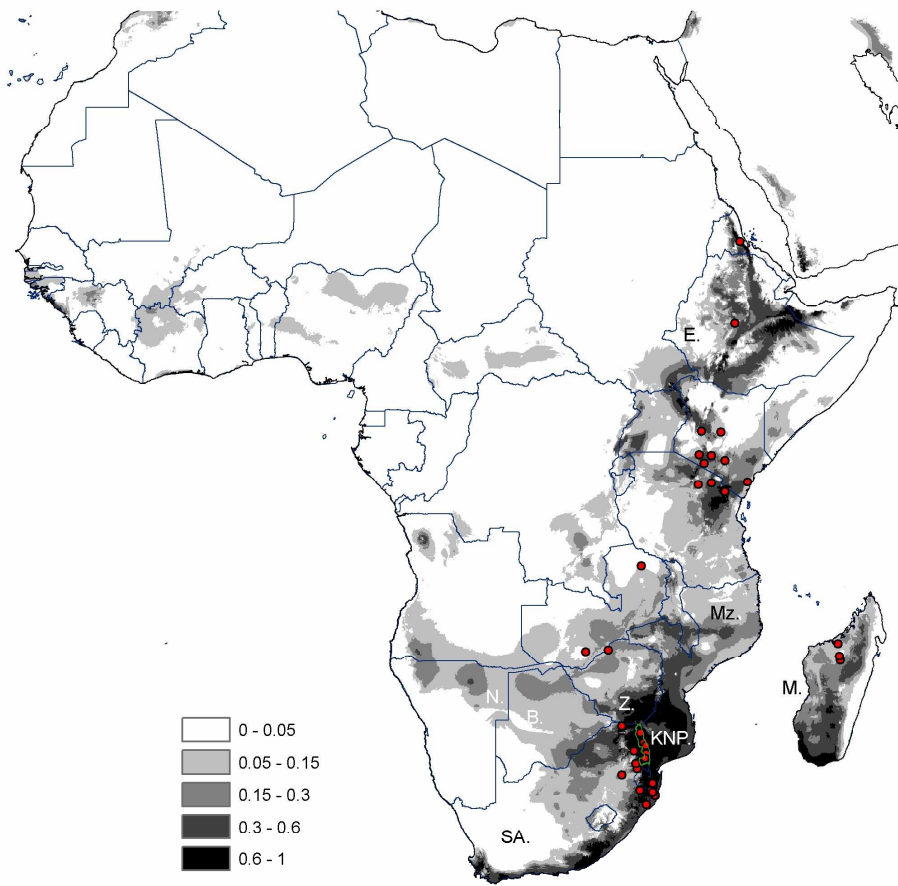


Figure1c

Fig. 2.

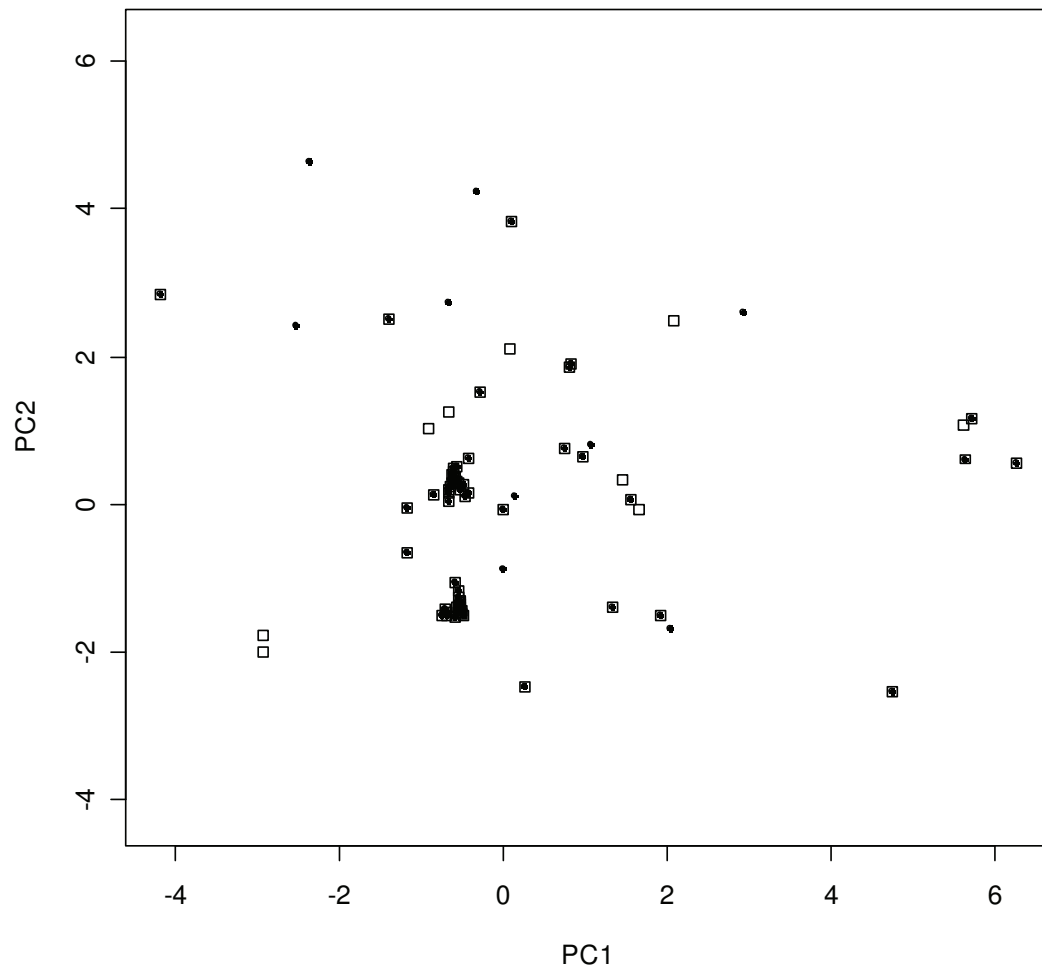


Fig. 3.

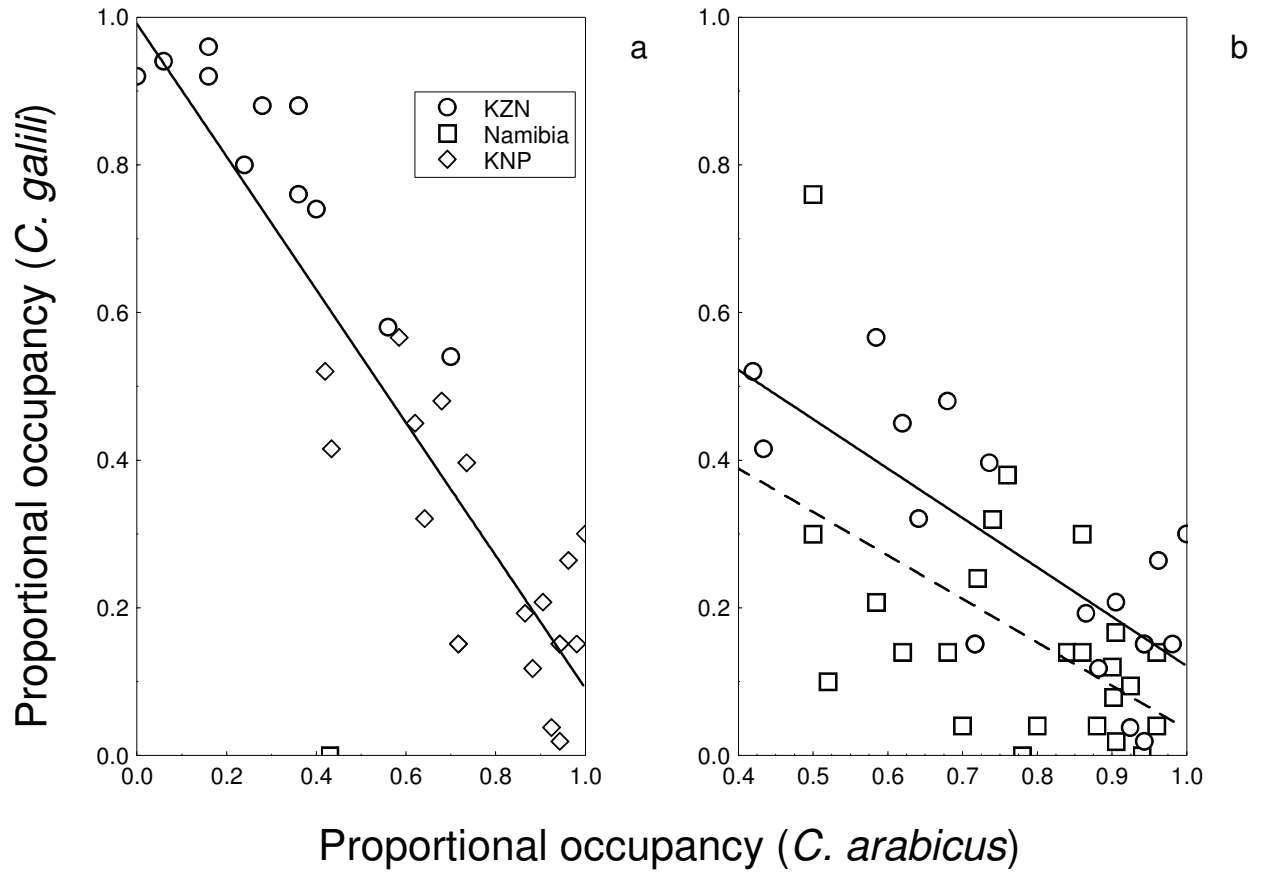


Fig 4.

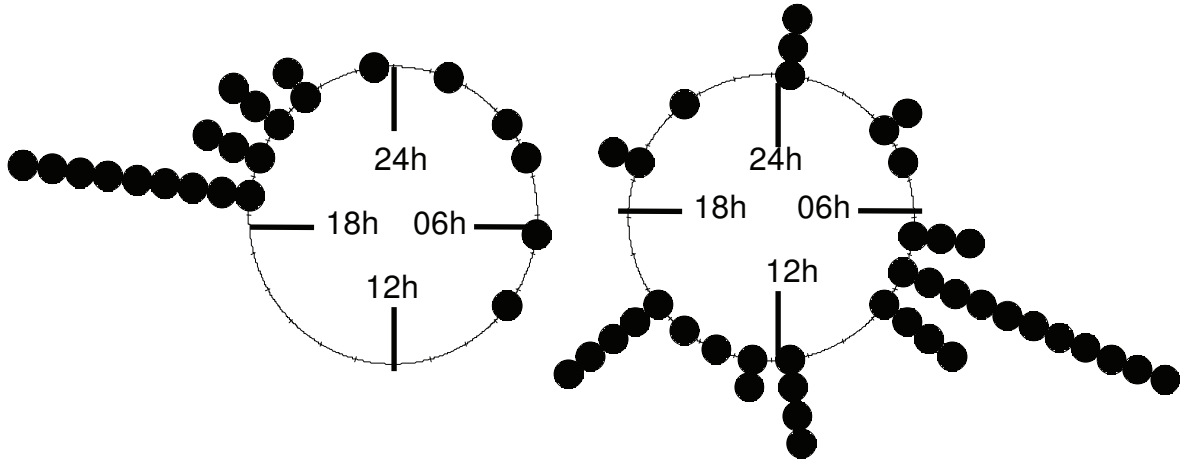


Fig 5.

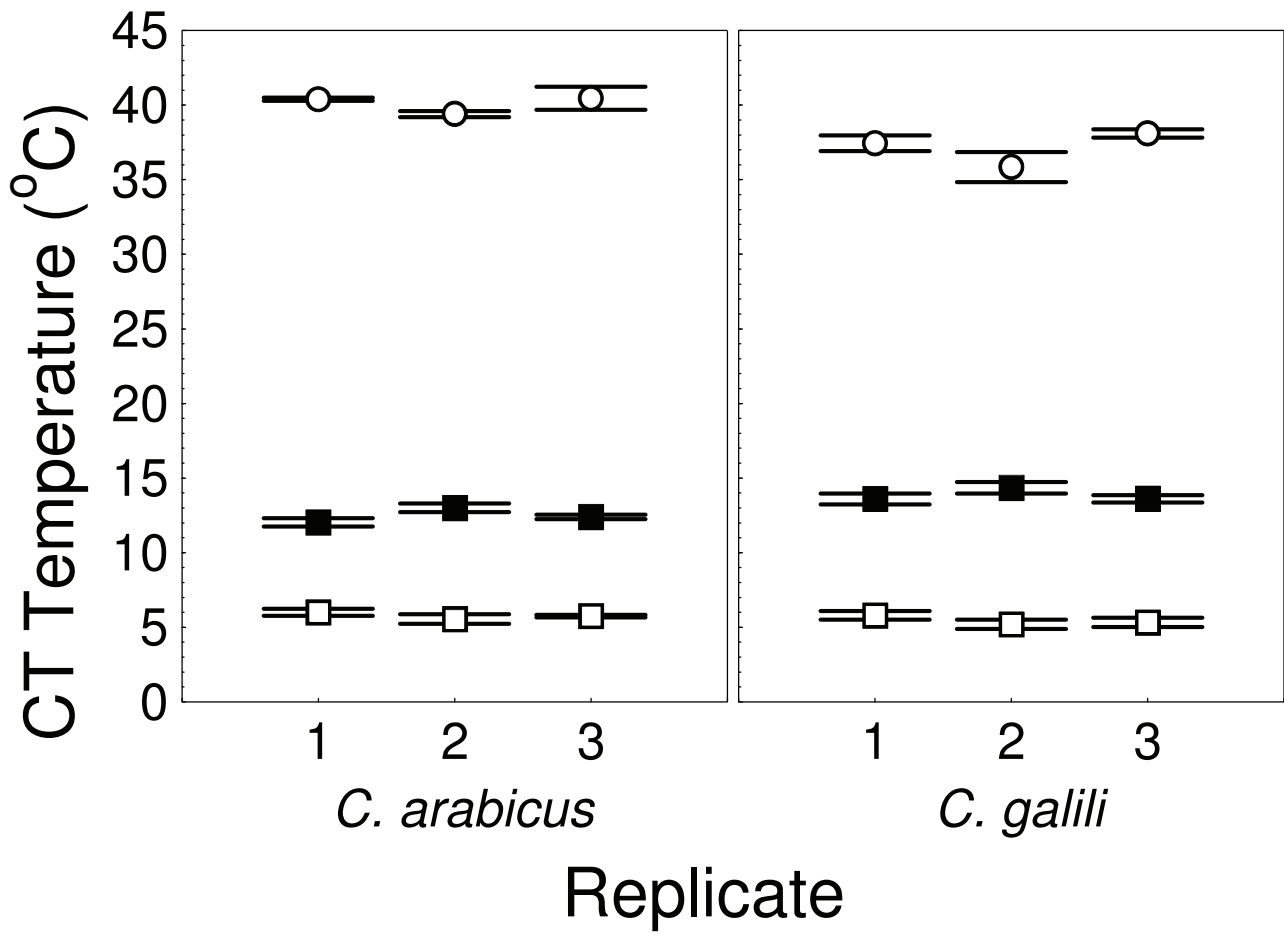


Fig. 6.

