

Do climatic conditions affect host and parasite phenotypes differentially? A case study of magpies and great spotted cuckoos

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Received: 27 February 2013 / Accepted: 2 September 2013 / Published online: 28 September 2013
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Abstract Climatic conditions, through their effects on resource availability, may affect important life history strategies and trade-offs in animals, as well as their interactions with other organisms such as parasites. This impact may depend on species-specific pathways of development that differ even among species with similar resource requirements (e.g., avian brood parasites and their hosts). Here we explore the degree of covariation between environmental-climatic conditions and nestling phenotypes (i.e., tarsus length, body mass, immune response to phytohemagglutinin injection) and ectoparasite loads of great spotted cuckoos (*Clamator glandarius*) and those of their magpie (*Pica pica*) hosts, both within and among 11 study years (1997–2011). Our main results were that (1) nestling phenotypes differed among years, but differently for great spotted

cuckoos and magpies; (2) nestling phenotypes showed significant among-year covariation with breeding climatic conditions (temperature and precipitation); and (3) these associations differed for cuckoos and magpies for some phenotypic traits. As the average temperature at the beginning of the breeding season (April) increased, body mass and tarsus length increased only for cuckoos, but not for magpie hosts, while immune response decreased in both species. Finally, (4) the strength of the within-year relationships between the probability of ectoparasitism by *Carnus hemapterus* flies and laying date (used as an estimate of the within-year variation in climatic conditions) was negatively affected by the annual accumulated precipitation in April. These results strongly suggest that variation in climatic conditions would result in asymmetric effects on different species with respect to the probability of ectoparasitism, immunity and body size. Such asymmetric effects may affect animal interactions in general and those of brood parasites and their hosts in particular.

Communicated by Indrikis Krams.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-013-2772-y) contains supplementary material, which is available to authorized users.

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Keywords Brood parasitism · *Carnus hemapterus* ·
Immunity · Laying date · Nestling growth

Introduction

To increase our knowledge of the effects of climatic conditions on ecological communities is currently a major topic in ecological studies mainly because it could help us to understand and predict effects of climatic change on natural populations and ecological interactions (White 2008; Boggs and Inouye 2012). Within this framework, it is particularly important to explore the effects of climatic conditions on the interaction between parasites and their hosts because of the intrinsic interest in understanding

how asymmetric selection pressures on hosts and parasites would affect their relationship (Merino and Møller 2010).

There are several reasons to expect different effects of variation in climatic conditions on parasites and their hosts. Parasites depend on their hosts for reproduction and small changes in the host's life cycle due to climatic conditions may, for instance, result in difficulties for parasites to find appropriate hosts (Saino et al. 2009), which in the case of consistent climatic change would produce a consistent change in selection pressure resulting in rapid changes in populations of parasites and hosts (Douglas et al. 2010; Møller et al. 2010). Once parasites have successfully reached their hosts, variation in resource availability due to climatic conditions (DeL Grosso et al. 2008; Studds and Marra 2011) would be relatively more important for hosts than for their parasites because the latter obtain resources directly from the former. Thus, it is likely that variation in climatic conditions affects parasites and their hosts at a different strength and therefore also how parasites and hosts interact (Kutz et al. 2005; Merino and Møller 2010; Martínez and Merino 2011).

Particularly important is the effect that climatic conditions may have on the immune system of animals, which is the primary line of host defense against pathogens. Development of the immune system as well as the immune response itself is energetically expensive (Råberg et al. 2000; Ots et al. 2001), and thus energetic deficit caused by a decrement in resource availability linked to climate conditions would restrict levels of host immune responses (Martínez and Merino 2011). Moreover, development of the immune system trades against growth (Soler et al. 2003), and thus variation in resource availability due to climatic conditions is expected to affect different aspects of the phenotypes of developing offspring either directly or indirectly through trade-offs between immunity and growth. In addition, climatic variation as such may also alter immune function and therefore the outcomes of host-parasite relationships. For instance, an increase in temperature may result in reduced innate and adaptive immune responses (Sinclair and Lochmiller 2000; Zahraa 2008), while for some taxa it may enhance the production of lysozyme and IgM (Chen et al. 2002; Ndong et al. 2007). Furthermore, intrinsic species-specific physiological characteristics related to growth and the immune system may interact with climatic conditions (see, Merino and Møller 2010).

Most animal parasites are also hosts for other parasites (Price 1980) and at a target level, climatic conditions may affect immune responses and other phenotypic traits of both parasites and hosts and, therefore, host–parasite interactions. A good example of this possibility may be found in the avian brood parasite–host systems. Obligate avian brood parasites lay their eggs in nests of host species, which incubate and take care of parasitic offspring until

independence (Roldán and Soler 2011). Brood parasitic and host chicks therefore develop in similar environments (i.e., nests, determined by the host species), depend on similar kinds of resources (i.e., a suitable diet) for their proper development (Grim and Honza 1997; Davies 2000; Yang et al. 2013), and also suffer from similar parasitic infections (Soler et al. 1999).

In this paper we explore and compare the effects of environmental conditions on growth and immunocompetence in nestlings of the brood parasitic great spotted cuckoo (*Clamator glandarius*) and its main host in Europe, the magpie (*Pica pica*). Several particularities of the great spotted cuckoo–magpie system make it appropriate for exploring this idea. First, the laying date of magpies is negatively related to breeding success and the nestlings' phenotypic quality (Sorci et al. 1997; De Neve et al. 2004), which justifies the use of the laying date of magpies as a variable related to within-breeding season variation in environmental conditions affecting magpie and great spotted cuckoo phenotypes (Birkhead 1991). Furthermore, we have supportive experimental evidence that immunocompetence and growth in magpies are competing for the same resources during development (Soler et al. 2003), and that variation in ecological conditions may affect the resolution of this trade-off (De Neve et al. 2007). Thus, the expectation that variation in ecological conditions among study years should result in differences in nestling growth and immunocompetence of magpie nestlings, but also of their brood parasite, is well supported.

To study possible interspecific differences in the effects of climatic conditions on nestling phenotypes, we first explore the association between phenotypes and laying dates within study years to determine the within-year effects of environmental conditions on developing magpies and great spotted cuckoos. Next, we analyze how these within-year relationships vary among years. Secondly, we investigate whether among-year variations in magpie and cuckoo phenotypes covaried with climatic conditions experienced in different years. As variables reflecting climatic conditions, we use average temperature and accumulated precipitation during April (i.e., the main time for egg laying and incubation) and May (the main time for hatching and nestling growth), which we assume to be related to resource availability [i.e., insects (Martínez et al. 1992; Soler et al. 1995)], for developing nestlings (Studds and Marra 2011). Detecting interspecific differences in the effects of laying dates, years, and climatic conditions on nestling growth, immunity and ectoparasite infestation [variables that predict the probability of recruitment in birds (Cichon and Dubiec 2005; Moreno et al. 2005; Møller et al. 2009)] would support the hypothesis that climatic conditions can have a species-specific impact on developing chicks and suggest that intrinsic species-specific factors should be

considered for predicting the effect of climate change on animal communities.

Materials and methods

Data for this study were obtained during 11 breeding seasons spread over a period of 15 years (1997–2011) in a magpie population breeding at Guadix (37°18'N, 3°11'W, southern Spain). The study area is located within a high-altitude plateau of approximately 1,000 m a.s.l. and the habitat consists of sparse vegetation that includes cultivated cereals (especially barley) and many groves of almond trees (*Prunus dulcis*), pines (*Pinus halepensis* and *Pinus pinaster*) and holm oak (*Quercus ilex*) in which magpies prefer to build their nests. Brood parasitism by great spotted cuckoos is common in the area [e.g., 26.6 and 41.2 % in 2000 and 2001, respectively (Martín-Gálvez et al. 2007)]. Host offspring usually did not survive in parasitized nests (Soler et al. 1996), while more than one cuckoo nestling usually fledges from naturally parasitized magpie nests [for further information on the system see Soler and Soler (2000)].

At the beginning of the breeding seasons, the end of March, we started searching for new magpie nests that, once found, were checked every 3–4 days to estimate the day that the first egg was laid (i.e., laying date). Magpies usually lay a clutch of seven eggs and lay one egg per day, which allowed us to estimate laying date of nests detected during egg-laying. Afterwards, nests were visited every second day during the following 7 days to detect brood parasitism. One day before the estimated hatching date (day 15 and 21 after incubation starts for great spotted cuckoo and magpie eggs, respectively) we visited magpie nests to record the hatching date of cuckoo and magpie nestlings. When nestlings were 15–16 and 17–18 days old in parasitized and unparasitized nests, respectively, we again visited the nests to record nestling weight [with a Pesola spring balance (accuracy 0.5 g)] and tarsus length (with a digital caliper to the nearest 0.01 mm). At these ages, we also injected the mitogenic phytohemagglutinin-P (PHA-P; Sigma Chemical) to estimate the immune response. Briefly, nestlings were subcutaneously injected in the right wing web with 0.5 mg of PHA dissolved in 0.1 ml of physiological saline solution (Bausch & Lomb). The left wing web was injected with 0.1 ml of saline solution and thus used as control response due to manipulation. The thickness of each wing web was measured at the injection site with a digital pressure-sensitive micrometer (model ID-CI012 BS, accuracy 0.01 mm; Mitutoyo) before and 24 h after the injection. We repeated measurements of each wing web three times and, since they were highly repeatable (Soler et al. 2003), mean values were used in subsequent analyses. We estimated the T cell mediated immune response or wing

web index as the change in thickness of the right wing web (PHA injection) minus the change in thickness of the left (control) one (Soler et al. 1999).

On the day of biometric measurements we also checked the nestlings for traces of the ectoparasite *Carnus hemapterus*, a hematophagous fly that commonly parasitizes magpie nests (Soler et al. 1999). In the European roller (*Coracias garrulus*), the infestation of *C. hemapterus* was positively related to a PHA-immune response and laying date (Václav et al. 2008). Parasite load was estimated on an ordinal scale with 0 indicating no flies and no direct evidence of parasitism, and 5 indicating more than 20 flies, and most of the skin surface of the wings and abdomen covered with blood and pellets from *Carnus* activity. Intermediate numbers corresponded to intermediate traces of parasitism.

Most magpies start to reproduce in April, and successful nestlings leave the nests at the end of May. Thus, as variables reflecting climatic condition for developing offspring, we used average temperature and accumulated precipitation of these months collected from datasets supplied by the Ministry of Environment of the Andalusian Government web page (http://www.juntadeandalucia.es/medioambiente/servtc5/sica/sima_av.jsp?web=0) for Guadix; when these data were not available, we used data from one or two nearby localities (Charches or Fifiñana; data are shown in Supplementary Appendix 1). Local temperature and/or precipitation predicts net primary production of the ecosystem (Del Grosso et al. 2008; Sala et al. 2012) as well as food availability for animals (White 2008) and developing nestlings (Studds and Marra 2011), which justifies the use of these variables.

Sample size and statistical analyses

During the 11 study years we sampled a total of 680 magpie nests, of which 162 were parasitized. All parasitized nests used in this study were naturally parasitized by the great spotted cuckoo in which no magpie offspring survived until biometric measurements. We did not use magpie nests with great spotted cuckoos and magpies growing together in the same nest because they are relatively rare in nature (Soler et al. 1996), and because nestling physical conditions can be directly influenced by the presence of nestlings of the other species in the nest (Soler and Soler 1991). The average number of magpie nestlings in non-parasitized nests was 3.63 (minimum = 1, maximum = 8), while that of cuckoos in parasitized nests was 1.62 (minimum = 1, maximum = 4). In 1998 and 2007 parasitized nests were used for other experiments and hence no data on great spotted cuckoos are available for these years. In addition, we only used nests of first breeding attempts of magpies that were not subjected to clutch- or brood-size

manipulations that are required for other experiments performed in our study population (e.g., Martín-Gálvez et al. 2012). For sample sizes per study year and species, as well as mean values for measured characters, see Supplementary Appendix 1.

After correcting for the significant effect of study year, among-nest variation in tarsus length, body mass, immune response and ectoparasite load was significantly larger than within-nest variation (see Supplementary Appendix 2). Thus, we used mean values per magpie nest for subsequent analyses. The within-season relationship between nestling phenotypes and laying dates was explored by means of partial correlation coefficients (Supplementary Appendix 1) of laying date after controlling for the effect of brood size. The effect of species identity on the relationship between laying date and nestling phenotype was tested by means of multivariate analyses of covariance (MANCOVAs) where tarsus length, body mass, immune response and parasite load were the dependent variables describing phenotypes of magpies and great spotted cuckoos; year, species identity and their interaction were included as fixed factors, and brood size (see below) and laying date as the continuous predictors. Squared laying date was not included in the model because we did not find evidence of a curvilinear relationship between nestling phenotypes and laying date after controlling for the effect of year, species identity and the linear association with laying date (see “Results”) (squared laying date effect: Wilks = 0.989, $F = 1.55$, $df = 4.578$, $P = 0.19$). Because of systematic differences in brood sizes between parasitized and non-parasitized nests, among-nest variation in brood size was corrected by using the residuals after correcting for identity of nestling species. A principal components analysis (PCA) was not a solution to reduce the number of variables because three factors were selected to explain 91 % of the variation in the four phenotypic variables. Hence we used multivariate ANOVAs to assess the predicted relationships on the four phenotypic variables together. To explore interspecific and among-year differences in slopes between laying dates and nestling phenotypic traits we ran additional MANCOVAs that also included the interactions between laying date and species identity, and between laying date and study year.

To determine the influence of climatic conditions on host and parasite phenotypes, we explored the relationships between least square means (i.e., controlled for brood size) of variables describing nestling phenotype (i.e., tarsus length, body mass, immune response to PHA, and parasite loads) estimated for each study year and variables describing atmospheric conditions (i.e., average temperature and accumulated precipitation in April and May). We also explored how climatic conditions of different study years affected the strength of the estimated intra-year partial correlations between phenotypic variables (corrected for

brood size) and mean laying date. These relationships were explored by means of MANCOVAs with the intra-year correlation coefficients between phenotypic variables and laying date as dependent variables, species identity as the fixed factor and temperature and precipitation as two continuous predictors. The interactions between continuous predictors and the fixed factor were included one by one in the model to explore between-species differences in the relationships with climatic conditions. The analyses exploring inter-year variation were weighed by number of nests sampled for each of the study years, but the df were adjusted to the number of years, to take into account heterogeneity in the quantity of data collected in different years.

Univariate results from MANOVAs were explored to detect which of the considered variable(s) defining nestling phenotypes was/were responsible for the multivariate result. All the statistical analyses were performed with STATISTICA version 10 software (Statsoft 2011).

Results

Relationships between laying dates and phenotypes of magpie and great spotted cuckoo nestlings

Within-year variation in laying date explained a significant proportion of the phenotypic variance (MANCOVA, effect of laying date: Wilks = 0.948, $F = 7.95$, $df = 4.578$, $P < 0.0001$), but the strength of the relationship differed for different study years (MANCOVA, interaction between study year and laying date: Wilks = 0.911, $F = 1.69$, $df = 322.10365$, $P = 0.009$). Univariate results helped to identify which of the phenotypic traits measured in great spotted cuckoos and magpies were responsible for these general trends. Except for parasite loads, all measured traits of nestlings varied with laying date (Table 1). While immune response increased during the breeding seasons, tarsus length and body mass decreased (laying date effect in Table 1; Fig. 1). Moreover, the effect of study year on the relationship between laying date and nestling phenotypes was statistically significant for tarsus length and marginally significant for immune response (effects of the interaction between laying date and study year in Table 1).

Interspecific differences

The detected relationship between laying date and nestling phenotype was similar for great spotted cuckoos and magpies (MANCOVA, interactions between species identity and laying date: Wilks = 0.99, $F = 1.29$, $df = 4.58$, $P = 0.27$). In accordance with this finding, univariate results confirm that none of the associations between laying

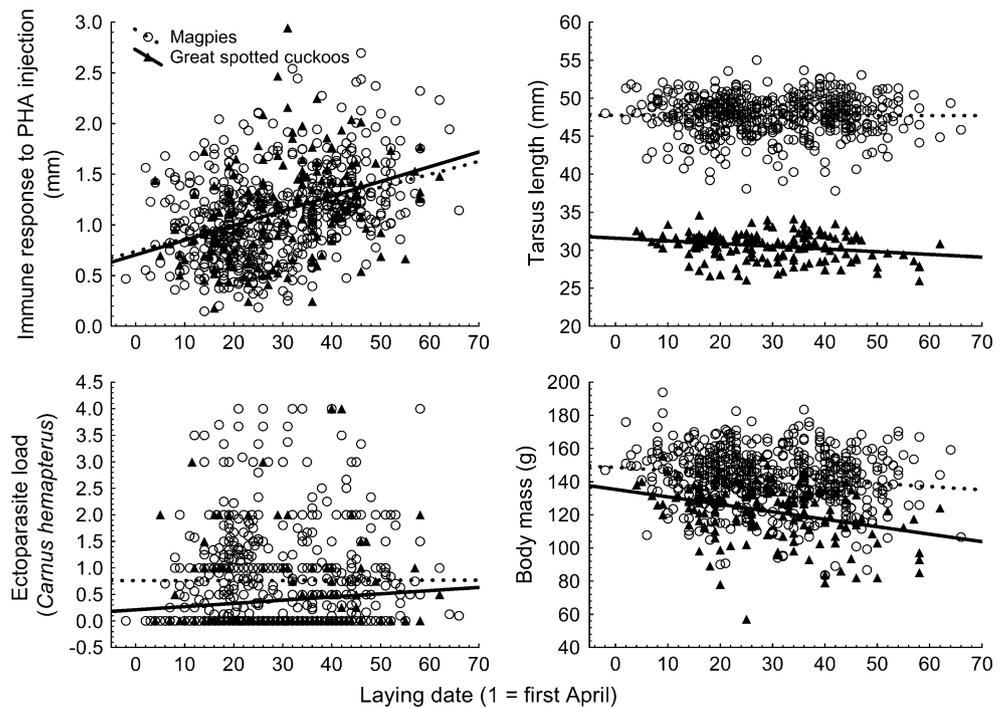
Table 1 Univariate results of the effects of laying date and brood size (covariates), species identity, study year (fixed factors) and the interactions between fixed factors explaining nestling phenotypes

[tarsus length, body mass, immune response to phytohemagglutinin (PHA) injections and parasite load of *Carnus hemapterus*]

	df	Tarsus length		Body mass		PHA response		Parasite load		
		F	P	F	P	F	P	F	P	
Laying date	1.581	19.35	<0.0001	15.93	0.0001	7.94	0.005	1.94	0.16	
Brood size	1.581	1.38	0.24	28.63	<0.0001	10.40	0.0013	3.23	0.072	
Species	1.581	4301.67	<0.0001	120.15	<0.0001	0.21	0.64	28.74	<0.0001	
Year	8.581	6.50	<0.0001	3.05	0.002	31.76	<0.0001	3.51	0.0006	
Species × year	8.581	1.61	0.12	2.16	0.028	2.95	0.003	2.72	0.006	
Including interactions with covariate ^a										
Species × laying date	1.580	0.13	0.72	0.01	0.93	1.64	0.20	3.51	0.061	
Year × laying date	8.573	2.59	0.009	1.26	0.26	1.80	0.075	1.53	0.14	
(Year × species) × laying date	8.573	1.59	0.074	1.26	0.21	1.61	0.056	1.23	0.24	

^a To estimate differences in the slopes between laying dates and nestling traits for different species and study years, interactions between laying date and fixed factors were included one by one in separate models

Fig. 1 Relationships between laying date and phenotypes [immune response to phytohemagglutinin (PHA) injections (mm), tarsus length (mm), body mass (g) and parasite loads (ordinal scale)] of great spotted cuckoo (filled triangles and dotted lines) and magpie (empty circles and continuous lines) nestlings. Lines are regression lines



dates and phenotypic traits significantly differed between great spotted cuckoos and magpies (although marginally significant for parasite loads, interaction between laying date and species in Table 1; Fig 1). Finally, inter-year variation in the relationship between laying date and phenotypes differ for different species (MANCOVA, interactions between species identity, year and laying date: Wilks = 0.85, $F = 1.33$, $df = 68.220384$, $P = 0.041$), which was mainly due to the effect on PHA response and tarsus length (interaction between laying date and species and years in Table 1).

Inter-year variation in phenotypes of magpie and great spotted cuckoo nestlings

Phenotypes of great spotted cuckoo and magpie nestlings differ among study years (MANCOVA, effect of year: Wilks = 0.59, $F = 10.21$, $df = 32.213354$, $P < 0.0001$), even after correcting for significant differences between phenotypes of magpie and cuckoo nestlings (see below). Univariate tests showed that inter-year differences were apparent for all measured traits (effect of species in Table 1; Fig. 2).

Fig. 2 Least square (i.e., corrected by the effect of laying date) mean values \pm 95 % confidence intervals per study year of immune response to PHA injections (mm), tarsus length (mm), body mass (g) and parasite loads (ordinal scale) of great spotted cuckoo and magpie nestlings

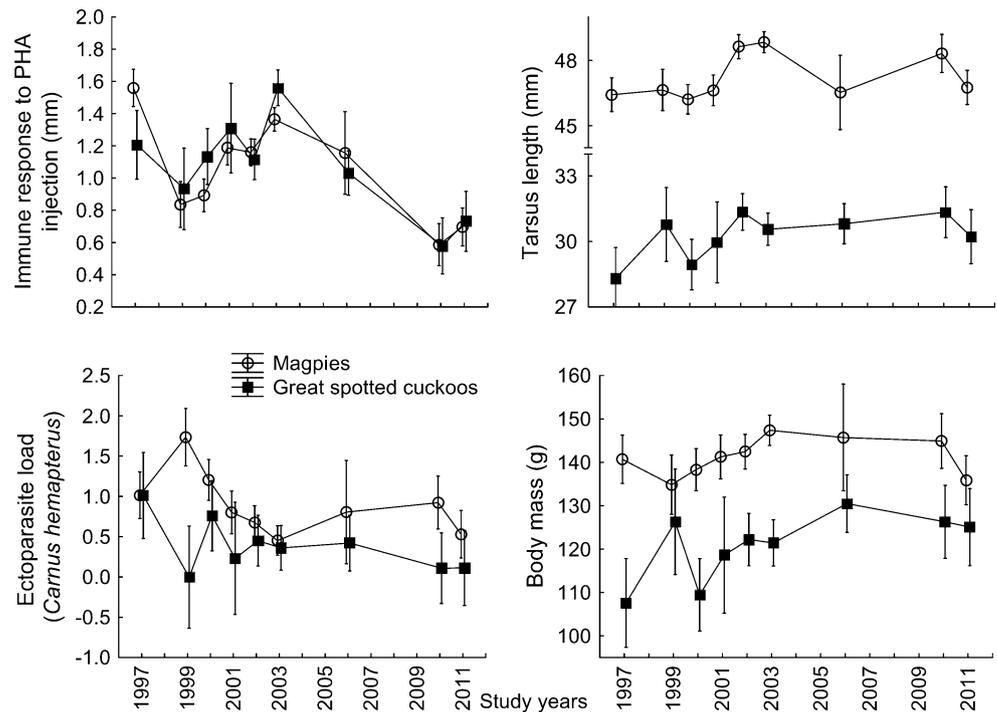
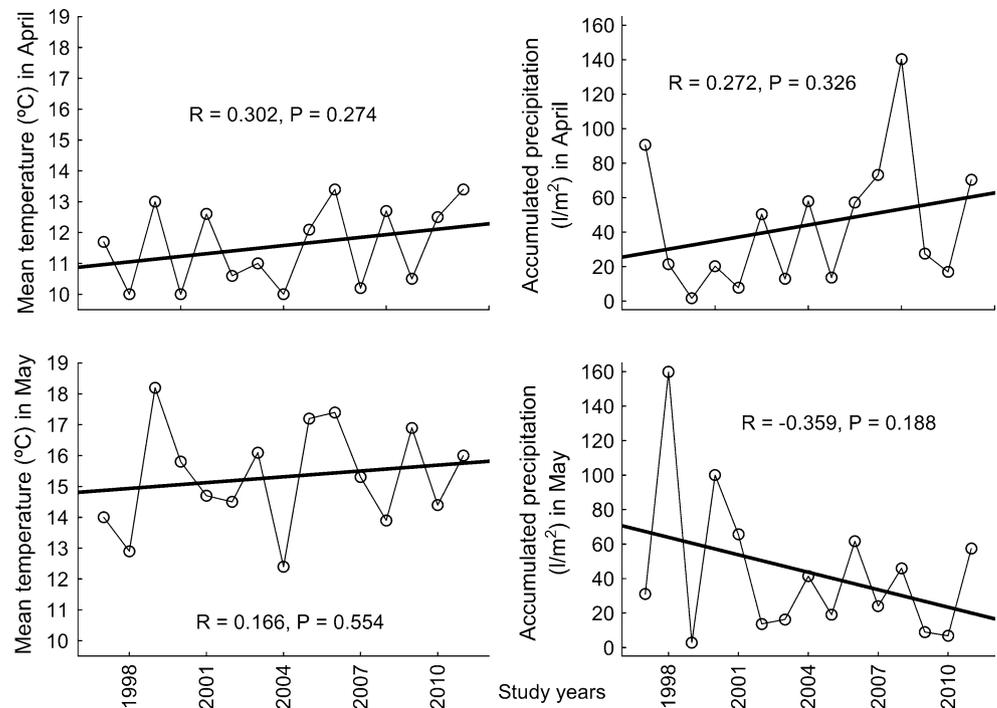


Fig. 3 Variation in climatic conditions [mean temperature ($^{\circ}$ C) and accumulated precipitation (l/m^2) in April and May] during the period 1997–2011. Correlation coefficients and the associated probability differing from zero are shown



Interspecific differences

Phenotypes of magpie and great spotted cuckoo nestlings differed significantly (Wilks = 0.07, $F = 1805.54$, $df = 4.578$, $P < 0.0001$). Except for the PHA response, which was similar in both species, magpie nestlings were heavier, had longer tarsi and on average experienced

a higher parasitism load than brood parasite nestlings (Table 1; Fig. 2).

The effect of study year on phenotypes varied for cuckoos and magpies (MANCOVA, interaction between study year and species: Wilks = 0.89, $F = 2.22$, $df = 32.213315$, $P = 0.0001$), which was mainly due to differential effects on PHA immune responses, parasite loads and body mass,

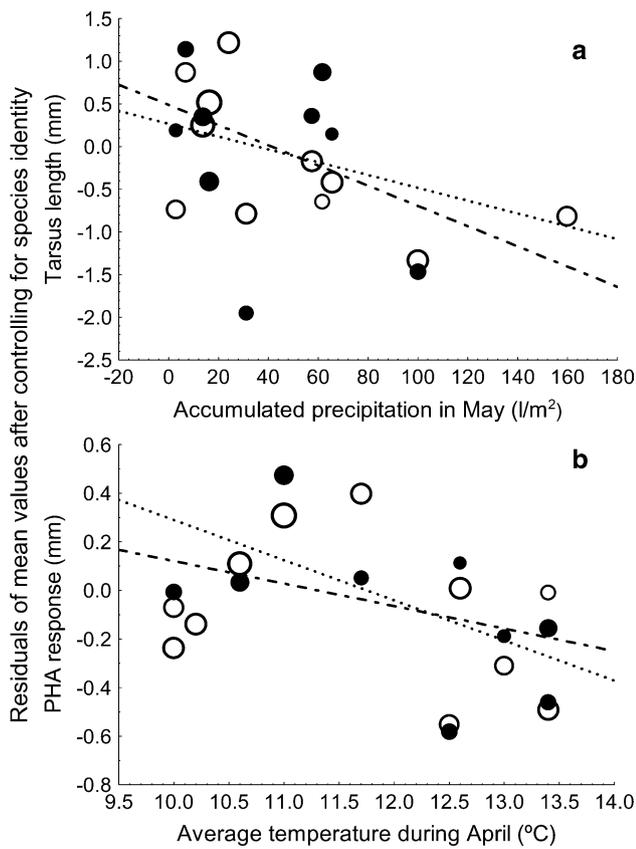


Fig. 4 Relationships between **a** accumulated precipitation in May (l/m^2) and tarsus length (mm) and **b** average temperature in April ($^{\circ}C$) and immune response to PHA injections (mm) in great spotted cuckoo (filled circles and dotted line) and magpie (open circles and dashed line) nestlings. Lines are weighted regression lines. Circle areas are proportional to the log-transformed number of nests that was sampled for each species in each study year

but not on tarsus length (species \times year interaction in Table 1; Fig. 2). In most years magpies were much more infested with parasites than cuckoos. In some years, great spotted cuckoos showed a stronger immune response than magpies, while in others years magpies showed the strongest immune responses (Fig. 2).

Variation in climatic conditions during the study period

Climatic conditions during the study period varied considerably but none of the trends between successive years and mean temperature or accumulated precipitation during April and May reached statistical significance (Fig. 3).

Among-year covariation between climatic conditions and phenotypes

After correcting for the effect of species identity (MANCOVA: Wilks = 0.001, $F = 2232.50$, $df = 4.11$, $P < 0.0001$), a significant proportion of among-year phenotypic variance was explained both by the average temperature in April (MANCOVA: Wilks = 0.33, $F = 5.50$, $df = 4.11$, $P = 0.011$) and by the accumulated precipitation in May (MANCOVA: Wilks = 0.157, $F = 14.75$, $df = 4.11$, $P = 0.0002$). None of the other climatic conditions explained among-year variation in phenotypes of nestlings (i.e., accumulated precipitation in April and average temperature in May; MANCOVA: Wilks > 0.89, $F < 0.35$, $df = 4.11$, $P > 0.84$). Univariate results indicated that the detected effects were mainly due to the negative associations between tarsus length and accumulated precipitation in May (Fig. 4a; Table 2) and immune response and average temperature in April (Fig. 4b; Table 2).

Table 2 Univariate results of the effects of climatic conditions [temperature (T), precipitation (P)] in different breeding seasons (covariate) and species identity (fixed factor), explaining inter-year vari-

ation in the nestling phenotypes (tarsus length, body mass, immune response to PHA injections and parasite load of *C. hemapterus*)

	<i>df</i>	Tarsus length		Body mass		PHA response		Parasite load		
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	
<i>T April</i>	1.14	0.02	0.89	1.60	0.23	3.50	0.082	0.83	0.38	
<i>P April</i>	1.14	0.02	0.89	0.30	0.59	0.20	0.66	0.33	0.57	
<i>T May</i>	1.14	0.03	0.86	0.06	0.81	0.00	0.97	1.09	0.31	
<i>P May</i>	1.14	6.21	0.026	0.02	0.89	1.81	0.20	0.04	0.85	
<i>Species</i>	1.14	1,819.35	<0.0001	50.02	<0.0001	0.12	0.73	4.73	0.047	
Including interactions with covariate ^a										
<i>T April \times species</i>	1.13	4.38	0.057	6.13	0.028	0.09	0.77	1.31	0.27	
<i>P April \times species</i>	1.13	0.02	0.88	0.01	0.99	0.06	0.82	0.28	0.61	
<i>T May \times species</i>	1.13	1.09	0.32	1.85	0.17	0.92	0.36	1.05	0.32	
<i>P May \times species</i>	1.13	0.14	0.72	0.04	0.85	0.07	0.80	0.09	0.77	

Terms with $P < 0.1$ in italics

^a To estimate differences in the slopes between climatic conditions and nestling traits for different species, the interactions between climatic conditions and the fixed factor were included one by one in separate models

Interspecific differences

In general, the effects of climatic conditions on phenotypes did not differ between the species (MANCOVA, interaction terms between climatic conditions and species: all Wilks > 0.69 , $F < 1.14$, $df = 4.10$, $P > 0.39$). Univariate results, however, showed that the effects of average temperature in April on tarsus length and body mass did differ for great spotted cuckoo (strongly positively related) and magpie (no relationship) nestlings (see interaction term in Table 2; Fig. 5).

Influence of climatic conditions on the relationship between laying date and phenotypes of magpie and great spotted cuckoo nestlings

After correcting for the non-significant effect of species identity (MANCOVA: Wilks = 0.64, $F = 1.52$, $df = 4.11$, $P = 0.26$), accumulated precipitation in April explained a significant proportion of among-year variance in the

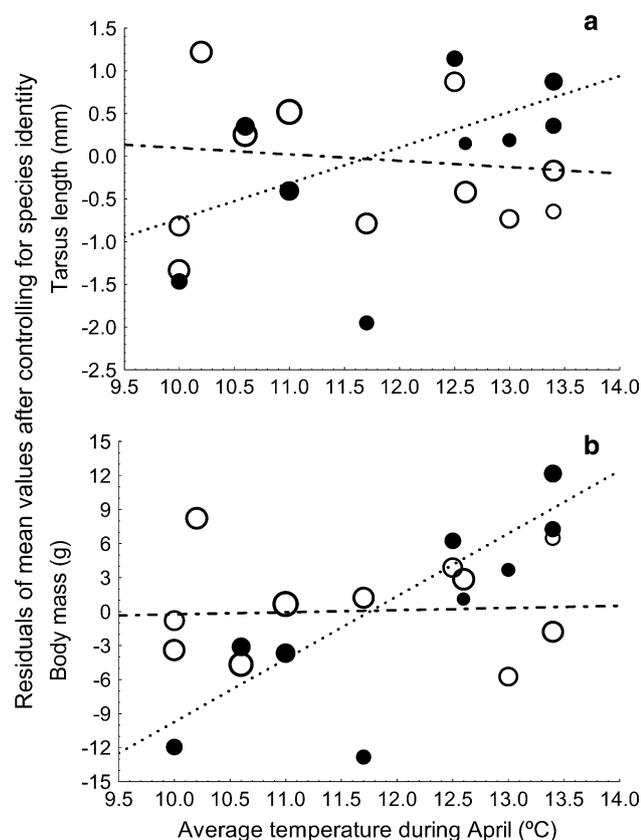


Fig. 5 Relationships between average temperature in April ($^{\circ}\text{C}$) and **a** tarsus length (mm) and **b** body mass (g) of great spotted cuckoo (filled circles and dotted lines) and magpie (open circles and dashed lines) nestlings. Lines are weighted regression lines. Circle areas are proportional to the log-transformed number of nests that was sampled for each species in each study year

strength of the relationship between laying date and phenotypes (MANCOVA: Wilks = 0.41, $F = 3.91$, $df = 4.11$, $P = 0.033$). This effect was mainly due to the significant negative association between precipitation in April and correlation coefficients between parasite loads and laying date and the marginally significant association between precipitation in April and correlation coefficients between PHA response and laying date (Table 3). In years of high precipitation during April, magpie and great spotted cuckoo nestlings from late broods suffered less from parasitism by *Carnus hemapterus* than early breeders, while the opposite occurred in years with little precipitation (Fig. 6a). A similar trend is observed for PHA responses (Fig. 6b). None of the other climatic factors in the MANCOVA model explained a significant proportion of variance (average temperatures in April and in May, and accumulated precipitation in May; MANCOVA: all Wilks > 0.64 , $F < 1.52$, $df = 4.11$, $P > 0.26$).

Interspecific differences

The effects of climatic conditions on the strength of the relationship between laying date and nestling phenotype marginally differ between cuckoos and magpies for precipitation in April (MANCOVA, interactions term: all Wilks = 0.46, $F = 2.97$, $df = 4.10$, $P = 0.074$) and temperature in May (MANCOVA, interactions term: all Wilks = 0.49, $F = 2.64$, $df = 4.10$, $P = 0.097$). No other measured climatic parameters approached statistical significance (MANCOVA, interactions term: all Wilks > 0.49 , $F < 1.57$, $df = 4.10$, $P > 0.10$). However, univariate results showed interspecific differences in these associations with some of the measured climatic parameters (interaction terms between species and climatic conditions in Table 3).

The relationship between the average temperature in April and the strength of the relationship between laying date and PHA response differed for great spotted cuckoo (no relationship) and magpie (negative relationship) nestlings (Table 3; Fig. 6b). In years of elevated temperature in April, magpie nestlings from late broods showed the weakest PHA responses, but in years of low temperature in April magpie nestlings from late broods showed the highest immune response (Supplementary Appendix 1). The strength of the association between laying date and PHA response for nestling cuckoos for different study years did not change in relation to the temperature in April (Supplementary Appendix 1). There was also a tendency for the relationship between accumulated precipitation in April and the strength of the relationship between body mass and laying date to differ between species (Table 3). The predicted negative effects of laying date on nestling development were stronger for cuckoos in dry years, but stronger for magpies in wet years (Fig. 6c) (Supplementary

Table 3 Univariate results of the effects of climatic conditions (T and P) in different breeding seasons and species identity (fixed factor), explaining inter-year variation in the strength (i.e., correlationcoefficients) of the relationships between phenotypes (tarsus length, body mass, immune response to PHA injections and parasite load by *C. hemapterus*) and laying date (see Supplementary Appendix 1)

	df	Laying date (R) Tarsus length		Laying date (R) Body mass		Laying date (R) PHA response		Laying date (R) Parasite load		
		F	P	F	P	F	P	F	P	
		<i>T April</i>	1.14	1.38	0.26	0.57	0.46	4.22	0.059	0.84
<i>P April</i>	1.14	1.77	0.20	0.40	0.54	1.87	0.19	9.10	0.009	
<i>T May</i>	1.14	1.94	0.19	1.05	0.32	0.04	0.85	3.22	0.09	
<i>P May</i>	1.14	0.02	0.90	0.56	0.47	0.60	0.45	0.03	0.86	
Species	1.14	1.33	0.27	0.69	0.42	0.06	0.82	2.68	0.12	
Including interactions with covariate ^a										
<i>T April × species</i>	1.13	0.16	0.70	1.37	0.26	8.28	0.013	0.78	0.39	
<i>P April × species</i>	1.13	0.39	0.54	5.02	0.043	0.03	0.86	0.17	0.69	
<i>T May × species</i>	1.13	0.63	0.44	0.05	0.82	4.24	0.060	0.13	0.72	
<i>P May × species</i>	1.13	1.14	0.30	1.64	0.22	0.90	0.36	4.27	0.059	

For abbreviations, see Table 2

Terms with *P*-values < 0.1 in *italics*^a To estimate differences in the slopes between climatic conditions and the strength of the relationships between phenotypes and laying date for different species, the interactions between climatic conditions and the fixed factor were included one by one in separate models

Appendix 1). Finally, the effect of precipitation in May on the strength of the relationship between laying date and parasite loads tended to differ for great spotted cuckoos and magpies (interaction terms in Table 3; Supplementary Appendix 1).

Discussion

Our main results were that:

1. The phenotype of nestlings differed among years and species, but not always in the same direction.
2. The within-year variation in environmental conditions (i.e., laying date) covaried with phenotypic traits and ectoparasitism in a similar way for great spotted cuckoo and magpie nestlings, although the strength of the relationships differed among years for some of the measured traits.
3. We found evidence of significant among-year covariation of nestling phenotypes and ectoparasitism with climatic conditions that, in some cases, differed for great spotted cuckoos and magpies. Given that great spotted cuckoo and magpie nestlings develop in similar environments (i.e., magpie nests), these results suggest that changing climatic conditions affect different species in different ways, and that these asymmetric effects may affect the relationship between brood parasites and their hosts.

Below we discuss the potential implication of these results within the following contexts: (1) asymmetric

effects of climatic conditions on different species, (2) possible interspecific differences in the resolution of the trade-off between immunity and growth, (3) the effect of climatic conditions on the probability that ectoparasitism may amplify environmental effects on interacting species, and (4) potential effects of climate change on the coevolutionary relationships between brood parasites and their hosts.

Asymmetric effects of climatic conditions on different species

Among-year variation in magpie and great spotted cuckoo phenotypes was especially apparent for parasite load and immune response. In general, magpies suffered more strongly from ectoparasites than cuckoos did, which is in accordance with previous findings and with the idea that brood parasites suffer less from selection due to parasitism than their hosts (Soler 1999; Soler et al. 1999). Interspecific differences in immune response were not consistent. Great spotted cuckoos showed stronger immune responses for some years while magpies did so in other years (Fig. 2). This result suggests that the rules which adjust the development of the immune system of great spotted cuckoos and of magpies to environmental conditions differed and, consequently, that their relative ability to counteract parasite infections may depend on environmental conditions. The detected covariation between phenotypes of magpies and great spotted cuckoo nestlings and climatic conditions of different study years are in accordance with this possibility. Since magpies and great spotted cuckoos grew in similar environments (i.e., magpie nests), these results suggest that

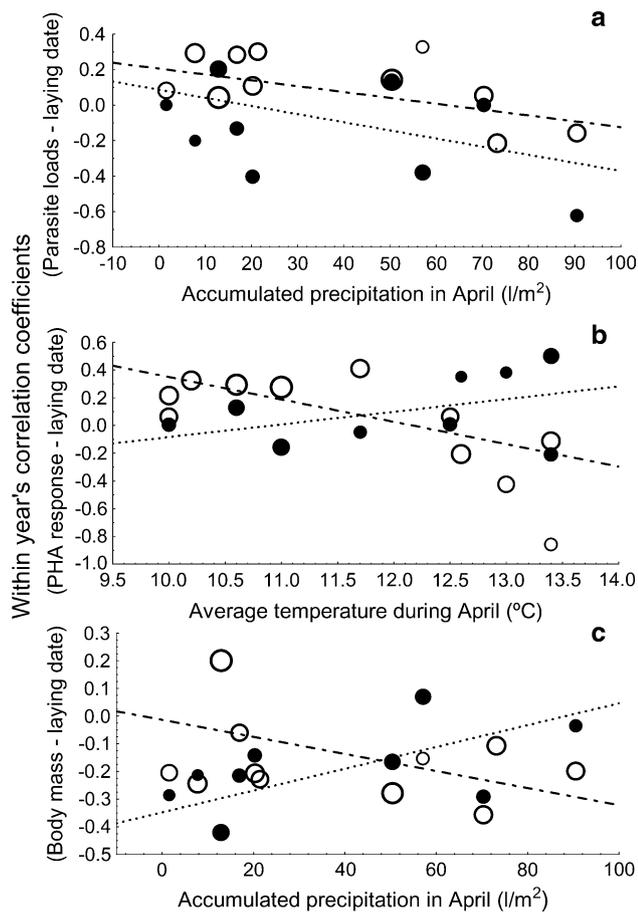


Fig. 6 Relationships between the accumulated precipitation in April (l/m^2) and **a** the strength (i.e., correlation coefficients) of the relationship between laying date and parasite loads and **b** body mass during different study years. The relationship between **c** average temperature in April ($^{\circ}C$) and the strength (i.e., correlation coefficients) of the relationship between laying dates and PHA responses of great spotted cuckoo (filled circles and dotted lines) and magpie (open circles and dashed lines) nestlings during different study years. Lines are weighted regression lines. Circle areas are proportional to the log-transformed number of nests that was sampled for each species in each study year

variation in climatic conditions may at least partially cause the detected covariation.

Environmental-climatic conditions and the resolution of the trade-off between immunocompetence and growth

As the breeding season progresses, the general pattern in temperate zones is that temperature and the probability of parasitism increases and resource availability for developing nestling decreases (Svensson 1997; Verboven and Visser 1998). Thus, because of the trade-off between immunity and growth (Soler et al. 2003), nestlings from late clutches might invest differentially more in antiparasitic defense at the cost of reduced growth rates (Merino et al. 2000), or

increase growth rates at the cost of reduced immunocompetence (Sorci et al. 1997; Saino et al. 1998) than nestlings from early clutches. In accordance with the former scenario, we found that the immune response of nestlings of both species increased, but that tarsus length and body mass decreased as the breeding season progressed (see Fig. 1). The strength, or even the sign, of the detected relationship between laying date and immunity, however, greatly varied among study years (see Supplementary Appendix 1; Fig. 6b, c), which may be explained by nestlings resolving the trade-off between growth and immunity depending on particular environmental conditions (i.e., resource availability, risk of parasitism, etc.) of different years. In agreement with this possibility we found that in years with higher mean temperatures, body mass and tarsus length of great spotted cuckoos increased and immune response decreased. For magpies, however, no such relationship appeared. Thus, these results suggest interspecific differences in the outcome of the trade-off favoring growth or immunity in relation to climatic conditions. Moreover, this interspecific difference may also help to explain the detected association between climatic conditions and bird phenotypes.

Environmental-climatic conditions, ecto-parasitism and the strength of the association between laying date and phenotypes

During dry years, cuckoo and magpie nestlings from late broods suffered more from ectoparasitism than those from early broods, while in wet years the opposite occurred (Fig. 6a). These results suggest that climatic conditions may affect *C. hemapterus*' traits that adjust the emergence from pupa to the reproduction phenology of their hosts (Valera et al. 2006; Calero-Torralbo et al. 2013). Thus, depending on year-specific climatic conditions, ecto-parasitism would mainly affect the early or the late breeders. While the laying stage of a female magpie is approximately 7 days, that of a great spotted cuckoo female may extend to about 2 months (Payne 1973), i.e., the complete breeding season of the magpie population. Thus, variation in the timing of emergence of *Carnus* ectoparasites in relation to climatic conditions would mainly affect individual magpies, which again implies asymmetric effects of climatic conditions on the reproductive success of magpies and great spotted cuckoos; in this case mediated by the effect of climatic conditions on the ectoparasite's breeding cycle.

Interestingly, it is known that birds adjust their laying date to environmental conditions including the probability of parasitism (Merino and Møller 2010). Here, we found that the probability of parasitism of nestlings from nests with different laying dates depended on year-specific climatic conditions. Consequently, it is possible that detected changes in laying dates of birds in relation to climate

change (Gordo and Sanz 2006) could not only be due to the direct effects of climate change on food availability, but also indirectly through the effects on the phenology of their ectoparasites.

Year-specific climatic conditions also tended to affect the strength of the relationship between immune response or body mass and laying date in cuckoos and magpies differently (Fig. 6b, c). Laying date, immunity and body mass are all variables that explain the probability of recruitment of birds (Møller and Saino 2004; Moreno et al. 2005; Cichon and Dubiec 2005) and, thus, these results suggest that climatic conditions would affect not only phenotypes, but also breeding parameters of magpies and cuckoos differentially.

Potential effects of climate change on the coevolutionary relationships between brood parasites and their hosts

Great spotted cuckoo and magpie nestlings experience similar environmental conditions during growth, and are involved in antagonistic coevolution. We have detected different effects of climatic conditions on body mass, tarsus length and immune response of cuckoos and magpies. All these phenotypic variables are good predictors of the probability of recruitment in birds (Møller and Saino 2004; Moreno et al. 2005; Cichon and Dubiec 2005). Avian brood parasites differ from their hosts in a large number of life history traits that affect coevolutionary relationships (Krüger and Davies 2002). It has been suggested that the effect of climatic conditions on laying date of hosts (Saino et al. 2009), or on egg coloration of hosts and brood parasites (Avilés et al. 2007) may affect the interactions between brood parasites and their hosts. Here, we found evidence suggesting differential effects of climatic conditions on variables related to survival of cuckoos and hosts. Hence, our results strongly further suggest that climatic conditions may affect the coevolutionary relationships in which magpies and great spotted cuckoos are engaged (Soler et al. 2001).

Acknowledgments We thank Anders Pape Møller, Manuel Soler, Gustavo Tomás and three anonymous reviewers for comments on previous versions of the manuscript that greatly improved the quality and clarity of the final version. Juan Manuel Peralta, Juan Gabriel Martínez and Gustavo Tomás helped at different stages of the field work. Funding was provided by the Spanish Ministerio de Ciencia e Innovación and European funds (FEDER) (CGL2010-19233-C03-01). Permission for sampling magpie nests and nestlings was obtained from the Junta de Andalucía (Spanish regional government of Andalucía).

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