Working paper

Wing shape and environmental energy are associated with molecular evolutionary rates in a large avian radiation

David Alejandro Duchêne[a,1], Paola Montoya[b,c], Carlos Daniel Cadena[b]

[a]Research School of Biology, Australian National University, ACT 2601, Australia
[b]Departamento de Ciencias Biológicas, Universidad de los Andes, Apartado 4976, Bogotá, Colombia
[c]Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Bogotá, Colombia

[1]Corresponding author
David A. Duchêne
Research School of Biology
Robertson Building, 46
Australian National University
Canberra, ACT 2601
Australia
Telephone: +61 4 12026379
Email: david.duchene@anu.edu.au

Abstract

Among the macroevolutionary drivers of molecular evolutionary rates, metabolic demands and environmental energy have been a central topic of discussion. The large number of studies examining these associations have found mixed results, and have rarely explored the interactions among various factors impacting molecular evolutionary rates. Taking the diverse avian family Furnariidae as a case study, we examined the association between several estimates of molecular evolutionary rates with a proxy of metabolic demands imposed by flight (wing morphology) and proxies of environmental energy across the geographic ranges of species (temperature and UV radiation). We found a strong positive association between molecular rates in genomic regions that can change the coded amino-acid with wing morphology, environmental temperature, and UV radiation. Strikingly, however, we did not find evidence of such associations with molecular rates at sites not impacting amino-acids. Our results suggest that the demands of flight and environmental energy primarily impact genome evolution by placing selective constraints, instead of being associated with basal mutation rates.

Keywords

Furnariidae, flight ability, hand-wing index, environmental temperature, environmental UV radiation

Introduction

The factors driving molecular evolution are a long standing matter of interest in the biological sciences. Some of the most studied factors correlating with rates of molecular evolution on a macroevolutionary scale include life-history traits (Bromham 2011), metabolic activity (e.g., Garcia-Porta *et al.* 2019) and environmental energy (Wright *et al.* 2006). These factors are also likely to interact, with the evolutionary outcome depending on the balance between the costs of sources of deleterious mutations and the requirements of the particular lifestyle of species (Bromham 2009). Although several studies have shown interactions among multiple correlates of molecular evolution (Lanfear *et al.* 2013; Bromham *et al.* 2015), there are gaps in our knowledge regarding the universality and relative contribution of different biological and environmental factors to molecular evolutionary rates.

Metabolic rate is an important biological driver of molecular evolution because metabolic activity produces mutagens (Gillooly *et al.* 2005; Allen *et al.* 2006). Specifically, metabolism produces oxygen and nitrogen free radicals (Barja 1999) which may cause novel mutations through damage to DNA (Cooke *et al.* 2003). Mass-specific metabolic rate is inversely associated with body mass (Martin & Palumbi 1993), which provides one explanation for the accelerated rates of molecular evolution in small vertebrates compared to large ones. While there is some evidence that metabolic rates are indeed associated with rates of molecular evolution (Bleiweiss 1998; Gillooly *et al.* 2005, 2007; Garcia-Porta *et al.* 2019), multiple studies have not found such an association (Mooers & Harvey 1994; Bromham *et al.* 1996; Lanfear *et al.* 2007; Galtier *et al.* 2009; Rolland *et al.* 2016).

An explanation for the mixed evidence of the impact of metabolism on molecular evolution is that there has been a heavy focus on body size and temperature as proxies of metabolic rate (Gillooly *et al.* 2001), yet multiple biological factors can impact metabolic requirements. For example, flight demand in birds is an extreme form of endurance exercise (Weber 2009), associated with high metabolic rate (Butler & Woakes 1990; Suarez 1992; Ward *et al.* 2001, 2002), high respiratory rate (Guglielmo *et al.* 2002), high rates of catabolism of lipids and proteins (Jenni-Eiermann *et al.* 2002), and oxidative stress (Jenni-Eiermann *et al.* 2014). Among the adaptations of particular avian lineages to sustain long periods of flight with high efficiency are also a high capillary density in flight muscles (Bishop *et al.* 1995; Maillet & Weber 2007), low wing loading (Norberg 1995; Alerstam *et al.* 2007), and a high hand-wing index (Dawideit *et al.* 2009). The high exposure to oxygen and nitrogen reactive species in birds with long-distance flight (Jenni-Eiermann *et al.* 2014) might have a mutagenic effect that leads to faster rates of molecular evolution beyond the effects of temperature and body mass.

Another widely studied factor that might drive molecular evolutionary rate is environmental energy through its mutagenic effect on the genome (Martin & Palumbi 1993; Allen *et al.* 2006). Studies in organisms including plants (Davies *et al.* 2004; Wright *et al.* 2006), marine organisms (Wright *et al.* 2010b), and lizards (Garcia-Porta *et al.* 2019) have all found associations between environmental energy and molecular rates. The link between energy and molecular evolution is often inferred using environmental temperature as a proxy of available energy (Wright *et al.* 2006). Other studies have focused on UV radiation (e.g., Davies *et al.* 2004), which is a well understood mutagen (Pawlowski *et al.* 1997). However, it is unclear how UV might impact the germline in many organisms, like endothermic vertebrates.

Environmental energy might also affect molecular evolution by mediating the fate of novel mutations, as opposed to affecting basal mutation rates. For example, temperature might influence the fitness effects of mutations, with maximal fitness occurring at around global maximal temperatures (Puurtinen *et al.* 2016). Energy might also facilitate the accumulation of biomass and allow for larger numbers of individuals to exist in populations (Wright 1983; Currie 1991; Willig *et al.* 2003); in larger populations, selection is more efficient and novel beneficial mutations are more likely to reach fixation rapidly (Kimura 1968; Ohta 1992; Lanfear *et al.* 2014). High energy might also lead to reduced investment in thermoregulation, allowing limited resources to be used in other activities, such as reproduction, and ultimately allowing for novel adaptations (Turner *et al.* 1988).

We examined the association between the rates of molecular evolution and the metabolic demands imposed by flight and environmental energy across species of an avian family. The avian family Furnariidae is a large radiation that includes the Neotropical ovenbirds and woodcreepers (Sibley & Monroe 1990; Remsen Jr *et al.* 2012). They have undergone a fast and consistent diversification over the last 30My (Derryberry *et al.* 2011) and inhabit a broad range of habitats of south and central America (Claramunt 2010). The species richness of the family and diversity of adaptations across broad gradients of altitude, latitude, and environmental conditions make it an interesting group for testing the association between molecular evolution and a range of adaptations (Fjeldså *et al.* 2005).

We focus on wing shape and environmental temperature and UV radiation as proxies of metabolic demand and environmental energy, respectively. Using data for the majority of species of Furnariidae (80%) we provide the first near-complete species-level examination of the correlates of molecular evolution in a large vertebrate radiation. We tested the association that wing shape and environmental energy have with molecular evolutionary rates in a set of nuclear and mitochondrial genomic regions. Individual tests across codon positions were made to dissect the independent effects on molecular signatures of selection or population size and mutation rate (Lanfear *et al.* 2010). Our data show that wing morphology and UV radiation are associated with molecular signatures of diversifying selection or small population sizes, suggesting an important impact of flight demands and environmental energy on the genome whilst not necessarily influencing the basal rate of mutation.

Methods

Data collection

We used published data on hand-wing index obtained from museum specimens for 280 species in Furnariidae (Claramunt *et al.* 2012b). The hand-wing index (Kipp 1959) is a metric of wing shape calculated as the length between the tip of the longest primary wing feather to the tip of the first secondary feather, divided by the wing chord (Claramunt *et al.* 2012b). Wing shape serve as a proxy of the demands of powered flight because they are critical for long distance bouts of flight (Pennycuick 2008; Claramunt *et al.* 2012b) and are associated with dispersal ability (Lockwood *et al.* 1998; Burney & Brumfield 2009; Dawideit *et al.* 2009). We thus assume that birds with a greater hand-wing index undergo greater amounts of flight, and hence experience greater metabolic demands associated with flight which may influence rates of molecular evolution. Data body mass was also collected for species of Furnariidae from a published compilation (Dunning 2008).

We collected data on environmental temperature and UV across the ranges of the species in the family Furnariidae. For all species, we downloaded georeferenced records from GBIF (Global Global Biodiversity Information Facility, https://www.gbif.org/) and VertNet (http://portal.vertnet.org/), discarding duplicated records and those outside the known distribution range for each species according to expert maps (del Hoyo *et al.* 2016). Using 145216 depurated records (mean per species=~615), we estimated the mean value for the annual mean temperature from WorldClim data (Fick & Hijmans 2017) at 30 arc-sec resolution, and for the annual mean UV-B from gIUV (Beckmann *et al.* 2014) with 15 arc-min resolution. Since current geographic distributions can differ greatly from the distributions in the past, contemporary measurements of environmental variables can introduce noise to subsequent phylogenetic regression analyses. This noise is likely to increase the Type II error rate but it is unlikely to cause a bias that increases Type I error rate, so the analyses of these type of data are in fact conservative (Davies *et al.* 2004).

Genomic data were taken from a published phylogenetic study of Furnariidae that included every species and mitochondrial and nuclear markers (Derryberry *et al.* 2011). Using data from three nuclear and three mitochondrial loci from the source phylogenetic study of Furnariidae, we identified species in Furnariidae for which data were available for environment, hand-wing index, and molecular evolutionary rates ($N = 236$). The molecular data and variables used in subsequent regression analyses are available online (github.com/duchene/furnariidae_rates).

Estimates of rates of molecular evolution

Relative rates of substitution across branches were estimated using Bayesian inference implemented in BEAST v2.4 (Bouckaert *et al.* 2014). We used a GTR+Γ substitution model (Tavaré 1986) and the uncorrelated log-normal relaxed clock model of substitution rates across branches (Drummond *et al.* 2006). We tested for potentially biased inferences from the substitution model using the tests of model adequacy in the software PhyloMAd (Duchêne *et al.* 2018). This assesses whether empirical data adhere to the assumptions made by the model by comparing them with data simulated under the model.

Substitution and clock model parameters were estimated independently for mitochondrial and nuclear data, as well as independently for 1st+2nd codon positions (CP12) versus 3rd codon positions (CP3) for the coding regions available. Rates of substitutions per unit of relative time across taxa were estimated using the phylogeny of genera extracted from the original phylogenetic study. We fixed the mean rate at CP3 sites at 1. The rates of CP12 sites were estimated relative to those at CP3 sites. The joint prior on the tree topology and relative node times was generated using a birth-death process. Posterior distributions were estimated by running analyses of 5×10^7 Markov chain Monte Carlo steps, with samples drawn every 5×10^3

steps. We discarded the first 10% of samples as burn-in and assessed satisfactory convergence of the posterior to a stationary distribution by verifying that the posterior of every sampled parameter had an effective sample size above 200. We made an independent replicate of each analysis to confirm that parameter estimates converged on the same results, and were not the product of reaching local optima. The median relative rate estimates of terminal branches across samples from the posterior distribution of each were used as rate estimates across species.

Regression analyses

We used phylogenetic generalized least squares (PGLS) regression models to examine the contribution of wing shape (a proxy for metabolic rate) and variables describing environmental energy to molecular evolutionary rates. Hypotheses were tested independently using nuclear and mitochondrial data, and using CP12 and CP3 rates as response variables. Estimates of CP3 rates largely represent molecular changes that do not influence the amino acid being coded (69%, 113/163), so they primarily represent the mutation rate (Kimura 1968). Meanwhile, CP12 rates mostly represent amino-acid substitutions (98%, 318/326), and are thus susceptible to the mutation rate and the interaction between selection and population size (Ohta 1992).

Because rates at CP12 and CP3 are in part driven by different processes, the relationship between the two provides additional information. The ratio between the two rates provides an indication of rate of fixation relative to the rate of mutation, effectively cancelling out the mutation rate and leaving the signal of the interaction between selection and population size (Ohta 1992). For this reason, we also included models where the response variable was the ratio between CP12 and CP3 rates. A high ratio can be a signature of historically small or fluctuating population sizes, or it can indicate positive selection at the genomic loci examined. A low ratio between rates can indicate the opposite historical signatures (i.e., large population sizes and/or purifying selection).

We tested a single regression model for each variable of molecular evolution as response. The explanatory variables in the model included the hand-wing index, environmental temperature, and UV radiation. We also included body mass as an explanatory variable due to its possible confounding effect of the association between the hand-wing index and molecular evolution. In each model we also included two interaction terms that represented the possible interactions among physiological surrogates and those among environmental variables. The interaction terms therefore included that between body mass and hand-wing index, and the interaction between the environmental energy and UV index.

To correct for non-independence due to relatedness between taxa, we used a species-level phylogenetic estimate extracted from the original phylogenetic study (Derryberry *et al.* 2011). We performed a transformation to many of the variables to adhere to least squares model assumptions (Supplementary Table S1). In PGLS, variables are assumed to have evolved along the phylogeny under a specified model of trait evolution. We used the lambda model of trait evolution (Freckleton *et al.* 2002), which was the model with the best fit to our data compared with three other common models (Brownian motion, kappa, and an Ornstein-Uhlenbeck process, results not shown).

The parameters of PGLS regression models were optimized using the *phylolm* R package (Ho & Ané 2014), and residuals were assessed for normality for each model. If no two-way interactions were significant, the models were executed without these interactions. If this model reduction did not significantly affect model fit according to a likelihood ratio test, then we also interpreted the parameter estimates from the reduced model.

Results

We found that the hand-wing index and variables of environmental energy are positively associated with molecular evolutionary rates, but not with the proxies of molecular evolution that are most closely associated with mutation rates (CP3 which largely do not change amino-acids). The overall molecular rates across all data are positively associated with hand-wing index ($P < 0.001$), environmental temperature (0.015), and UV radiation ($P < 0.001$; Table 1). Neither of the two regression interaction terms were found to significantly explain overall molecular rates.

The evolutionary rate at the regions in the mitochondrial data that mostly change amino acids (CP12) are associated with a negative interaction between body mass and hand-wing index ($P < 0.001$), which means that hand-wing index has a positive association with these molecular rates primarily in small-bodied species (Table S1; Figure 1). Similarly, we found that the evolutionary rates at nuclear regions that mostly change amino acids are positively associated with the hand-wing index ($P = 0.038$). The proxies of molecular evolutionary rate that are primarily impacted by selection and population size (CP12 / CP3), to the exclusion of the mutation rate, were explained by UV radiation in both mitochondrial ($P < 0.001$) and nuclear data ($P = 0.043$). In mitochondrial data this variable was also explained by the hand-wing index ($P < 0.001$) and environmental temperature ($P = 0.026$). Strikingly, none of the regression terms explored explained our closest proxy to the mutation rate (CP3) in either mitochondrial or nuclear data (Table S1).

Table 1. Overview of the PGLS regression results for the models tested for each of the variables of molecular rates explored (rows). Shaded cells indicate terms that were not significant in regression analyses. See Table S1 for full details on the variables and regression models tested.

Molecular rate response regression terms	Explanatory regression terms					
	Body mass	Hand-wing index	Temperature	UV radiation	Body mass × Hand-wing index	Temperature × UV radiation
Overall molecular rate		√	√	√		
Mitochondrial CP12	√	√			√	
Mitochondrial CP3						
Mitochondrial CP12 / CP3		√	√	√		
Nuclear CP12		√				
Nuclear CP3						
Nuclear CP12 / CP3				√		

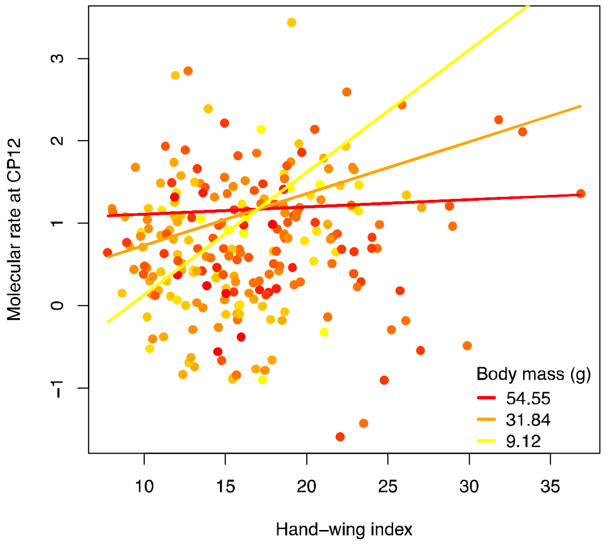

Figure 1. The association between mitochondrial molecular rates at the 1st and 2nd codon positions and hand-wing index across species of the avian family Furnariidae. Warmer colours indicate species with greater body mass. The predicted regression line is added for the mean body mass (orange), and each of the body masses one standard deviation from the mean (red and

yellow). Molecular rates are shown under a box-cox transformation; results with other transformations are qualitatively identical.

Discussion

Our study demonstrates that the wing morphology, environmental temperature, and UV radiation are positively associated with molecular evolutionary rates across the Neotropical avian family Furnariidae. Strikingly, however, we did not find such association in the regions of loci that largely reflect the mutation rate (i.e. substitution rate at the third codon position, CP3). The effects of metabolism and environmental energy on the genome might therefore not occur via the mutation rate, but instead through selective pressures or an effect on population size. This is consistent with previous studies showing that flight has led to a broad range of genomic as well as physiological adaptations (Tobalske *et al.* 2003; Wright *et al.* 2014). Our data are also consistent with evidence that metabolic rates do not dictate the basal rate of evolution across taxa (Lanfear *et al.* 2007), yet that environmental temperature is associated with molecular evolutionary rates in birds (Gillman *et al.* 2012). Future genome-scale studies will be instrumental to identify the selective role that the adaptation for flight and to high-energy environments have in driving whole-genome evolution. Recent examples include work on the selective impacts of skeletal structure and song on the genome (Zhang *et al.* 2014),

While theory predicts that metabolic rates can drive basal mutation rates (Allen *et al.* 2002; Gillooly *et al.* 2005), there are several reasons why this might not be the case. Most organisms exist at an upper limit of mutations, such that even a minor increase in mutation rate leads to an unsustainable loss in fitness (Bromham 2011). For example, large bodied mammals have a greater opportunity to accumulate mutations than small bodied mammals. This is because their long lifespan allows for more cell generations for producing gametes, which could interact with the smaller population sizes to result in a faster accumulation of deleterious changes (Bromham 2009). Nonetheless, instead of having a faster rate of accumulation of changes, large bodied mammals have slow molecular evolutionary rates that are possibly due to the increased selective pressures on DNA repair that avoids deleterious changes. Fast metabolic rates might pose a similar increased selective pressure for avoiding deleterious mutations, and might explain the several past studies that have not found an association between proxies of metabolic rate and those of mutation rates (e.g., Lanfear *et al.* 2007; Rolland *et al.* 2016). The impact of environmental temperature and UV radiation on mutation rates is also likely to be negligible in species that regulate their internal energy levels and whose gametes do not undergo direct exposure to environmental factors, such as most endotherms (but see Gillman *et al.* 2009). This contrasts with the impact that temperature can have on the mutation rates in organisms that are more exposed to environmental conditions, such as many marine taxa (Allen *et al.* 2006; Wright *et al.* 2010b), plants (Davies *et al.* 2004; Wright *et al.* 2006; Gillman *et al.* 2010), amphibians (Wright *et al.* 2010a), and reptiles (Garcia-Porta *et al.* 2019).

Independent sources of evidence show that metabolism has placed a set of selection pressures on bird physiology and genomics. For instance, powered flight requires high energy output (Suarez 1992) and efficiency (Kvist *et al.* 2001; Morris *et al.* 2010). The reduction in genome size seen in many avian taxa is likely to be an adaptation associated with the demands of powered flight (Wright *et al.* 2014). One explanation for our results showing increased rates in the genomic regions that are associated with selective pressures (CP12 and CP12/CP3) is that the demands of flight have favoured a broad range of evolutionary changes in the genome. Instead of placing increased pressure for the genome to remain unchanged, flight has allowed for changes in physiology and behaviour and remarkable opportunities of niche colonization (e.g., Gavrilov 2011; Claramunt *et al.* 2012a; Sol *et al.* 2012). Flight is a major driver of evolutionary change across the genome (Zhang *et al.* 2014), and this is supported by our results of an association between the hand-wing index and molecular evolution in both mitochondrial and nuclear regions.

Substitution rates at sites that undergo selection are also influenced by population size, often with a complex interaction between the two (Lanfear *et al.* 2014). For example, the relationship between population size and the substitution rate takes drastically different shapes depending on whether a genomic region is undergoing positive or negative selection. In cases of genomic regions that are largely undergoing negative selection, as is common in protein-coding loci, greater population sizes will increase the efficiency of selection and lead to a lower substitution rate, even under a constant selection coefficient. This means that our data cannot distinguish between an effect on selection or population size as caused by flight and environmental energy. Therefore, our results could be caused by a negative association between the population size and both the hand-wing index and environmental energy. However, evidence largely supports the hand-wing index as being associated with dispersal ability and larger effective population sizes (Claramunt *et al.* 2012b). Similarly, regions with greater environmental energy have been associated with greater accumulation of biomass (Mittelbach *et al.* 2007), so a negative association between our explanatory variables and population size seems unlikely. Instead, it is likely that the impact of these variables on the genome occurs entirely via patterns of selection.

Our results contribute to a body of work that suggests that the demands of flight and environmental energy are not necessarily associated with basal mutation rates, but that instead those factors have an impact on the genome through selection and adaptation. In birds, flight is associated with a range of adaptations that lead to positive selection in large swathes of the genome, and impact genome structure (Zhang *et al.* 2014). Similarly, adaptations to high energy environments might include direct adaptations to thermoregulate. More complex traits to survive in high-energy environments might be associated with the increased biotic interactions in these environments, such as competitive and pathogenic interactions (Schemske *et al.* 2009; Díaz *et al.* 2013). Data sets of whole genomes and detailed physiology across bird taxa (e.g., Jarvis *et al.* 2014; Sheard *et al.* 2019), will bring a more complete picture of the impact of metabolism and the environment on avian whole-genome evolution.

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Table S1. Complete results of PGLS regression models tested. Transformations used are shown beside variables in parentheses. The full model notation used in *phylolm* is shown, with successive model reductions indicated in letters (1a, 1b, etc.), where applicable.

1a

Overall molecular rate (log) ~ Body mass (Box-Cox) + Hand-wing index + Mean annual temperature + Mean UV radiation + Body mass (Box-Cox):Hand-wing index + Mean annual temperature:Mean UV radiation

	Estimate	Standard error	t-value	p-value
(Intercept)	-3.103	2.097	-1.480	0.140
Body mass (Box-Cox)	0.882	1.028	0.858	0.392
Hand-wing index	0.180	0.110	1.640	0.102
Mean annual temperature	0.016	0.032	0.479	0.633
Mean UV radiation	9.08×10^{-5}	7.38×10^{-5}	1.231	0.220
Body mass (Box-Cox):Hand-wing index	-0.051	0.056	-0.911	0.363
Mean annual temperature:Mean UV radiation	9.28×10^{-7}	5.72×10^{-6}	0.162	0.871

1b

Overall molecular rate (log) ~ Body mass (Box-Cox) + Hand-wing index + Mean annual temperature + Mean UV radiation + Body mass (Box-Cox):Hand-wing index

	Estimate	Standard error	t-value	p-value
(Intercept)	-3.105	2.092	-1.484	0.139
Body mass (Box-Cox)	0.847	1.003	0.845	0.399
Hand-wing index	0.176	0.107	1.649	0.101
Mean annual temperature	0.021	0.008	2.443	0.015
Mean UV radiation	1.02×10^{-4}	2.76×10^{-5}	3.688	<0.001
Body mass (Box-Cox):Hand-wing index	-0.049	0.054	-0.900	0.369

1c

Overall molecular rate (log) ~ Body mass (Box-Cox) + Hand-wing index + Mean annual temperature + Mean UV radiation

	Estimate	Standard error	t-value	p-value
(Intercept)	-1.457	1.013	-1.438	0.152
Body mass (Box-Cox)	0.017	0.394	0.043	0.966
Hand-wing index	0.081	0.014	5.866	<0.001
Mean annual temperature	0.021	0.008	2.439	0.015
Mean UV radiation	1.00×10^{-4}	2.76×10^{-5}	3.639	<0.001

2a

Molecular CP12 rate (Box-Cox) ~ Body mass (Box-Cox) + Hand-wing index + Mean annual temperature + Mean UV radiation + Body mass (Box-Cox):Hand-wing index + Mean annual temperature:Mean UV radiation

	Estimate	Standard error	t-value	p-value
(Intercept)	-8.091	2.320	-3.487	0.001
Body mass (Box-Cox)	3.489	1.138	3.066	0.002
Hand-wing index	0.496	0.122	4.075	<0.001
Mean annual temperature	0.044	0.036	1.233	0.219
Mean UV radiation	1.54×10^{-4}	8.17×10^{-5}	1.882	0.061
Body mass (Box-Cox):Hand-wing index	-0.218	0.062	-3.534	<0.001
Mean annual temperature:Mean UV radiation	-4.11×10^{-6}	6.34×10^{-6}	-0.648	0.518

3a

Molecular CP3 rate (Box-Cox) ~ Body mass (Box-Cox) + Hand-wing index + Mean annual temperature + Mean UV radiation + Body mass (Box-Cox):Hand-wing index + Mean annual temperature:Mean UV radiation

	Estimate	Standard error	t-value	p-value
(Intercept)	2.387	3.753	0.636	0.525
Body mass (Box-Cox)	1.105	1.840	0.601	0.549
Hand-wing index	0.134	0.197	0.681	0.496
Mean annual temperature	0.020	0.058	0.349	0.728
Mean UV radiation	3.84×10^{-5}	1.32×10^{-4}	0.291	0.771
Body mass (Box-Cox):Hand-wing index	-0.065	0.100	-0.653	0.515
Mean annual temperature:Mean UV radiation	-3.42×10^{-6}	1.02×10^{-5}	-0.334	0.739

3b

Molecular CP3 rate (Box-Cox) ~ Body mass (Box-Cox) + Hand-wing index + Mean annual temperature + Mean UV radiation + Body mass (Box-Cox):Hand-wing index

	Estimate	Standard error	t-value	p-value
(Intercept)	2.395	3.746	0.640	0.523
Body mass (Box-Cox)	1.234	1.796	0.687	0.493
Hand-wing index	0.149	0.191	0.776	0.438
Mean annual temperature	0.002	0.015	0.100	0.920
Mean UV radiation	-2.48×10^{-6}	4.95×10^{-5}	-0.050	0.960
Body mass (Box-Cox):Hand-wing index	-0.073	0.097	-0.759	0.449

3c

Molecular CP3 rate (Box-Cox) ~ Body mass (Box-Cox) + Hand-wing index + Mean annual temperature + Mean UV radiation

	Estimate	Standard error	t-value	p-value
(Intercept)	4.880	1.814	2.691	0.008
Body mass (Box-Cox)	-0.019	0.706	-0.027	0.978
Hand-wing index	0.005	0.025	0.187	0.852
Mean annual temperature	0.001	0.015	0.096	0.923
Mean UV radiation	-4.89×10^{-6}	4.93×10^{-5}	-0.099	0.921

4a

CP12/CP3 ratio (Box-Cox) ~ Body mass (Box-Cox) + Hand-wing index + Mean annual temperature + Mean UV radiation + Body mass (Box-Cox):Hand-wing index + Mean annual temperature:Mean UV radiation

	Estimate	Standard error	t-value	p-value
(Intercept)	-14.566	4.868	-2.992	0.003
Body mass (Box-Cox)	3.653	2.387	1.530	0.127
Hand-wing index	0.600	0.255	2.351	0.020
Mean annual temperature	0.020	0.075	0.265	0.791
Mean UV radiation	1.76×10^{-4}	1.71×10^{-4}	1.026	0.306
Body mass (Box-Cox):Hand-wing index	-0.244	0.129	-1.887	0.060
Mean annual temperature:Mean UV radiation	4.44×10^{-6}	1.33×10^{-5}	0.334	0.738

4b

CP12/CP3 ratio (Box-Cox) ~ Body mass (Box-Cox) + Hand-wing index + Mean annual temperature + Mean UV radiation + Body mass (Box-Cox):Hand-wing index

	Estimate	Standard error	t-value	p-value
(Intercept)	-14.577	4.859	-3.000	0.003
Body mass (Box-Cox)	3.486	2.330	1.496	0.136
Hand-wing index	0.581	0.248	2.339	0.020
Mean annual temperature	0.044	0.020	2.260	0.025
Mean UV radiation	2.29×10^{-4}	6.42×10^{-5}	3.566	<0.001
Body mass (Box-Cox):Hand-wing index	-0.234	0.125	-1.866	0.063

4c

CP12/CP3 ratio (Box-Cox) ~ Body mass (Box-Cox) + Hand-wing index + Mean annual temperature + Mean UV radiation

	Estimate	Standard error	t-value	p-value
(Intercept)	-6.648	2.367	-2.808	0.005

Body mass (Box-Cox)	-0.511	0.921	-0.555	0.580
Hand-wing index	0.121	0.032	3.775	<0.001
Mean annual temperature	0.044	0.020	2.238	0.026
Mean UV radiation	2.21×10^{-4}	6.44×10^{-5}	3.435	0.001

5a

Molecular CP12 rate (log) ~ Body mass (log) + Hand-wing index + Mean annual temperature + Mean UV radiation + logmass:Hand-wing index + Mean annual temperature:Mean UV radiation

	Estimate	Standard error	t-value	p-value
(Intercept)	-0.384	0.671	-0.572	0.569
Body mass (log)	-0.057	0.166	-0.340	0.735
Hand-wing index	-0.003	0.032	-0.088	0.931
Mean annual temperature	0.015	0.026	0.560	0.578
Mean UV radiation	4.69×10^{-5}	6.92×10^{-5}	0.677	0.501
logmass:Hand-wing index	0.005	0.009	0.478	0.634
Mean annual temperature:Mean UV radiation	-2.66×10^{-6}	4.74×10^{-6}	-0.562	0.576

5b

Molecular CP12 rate (log) ~ Body mass (log) + Hand-wing index + Mean annual temperature + Mean UV radiation + Mean annual temperature:Mean UV radiation

	Estimate	Standard error	t-value	p-value
(Intercept)	-0.614	0.463	-1.326	0.190
Body mass (log)	0.020	0.047	0.424	0.673
Hand-wing index	0.012	0.006	2.114	0.038
Mean annual temperature	0.013	0.026	0.498	0.620
Mean UV radiation	4.5×10^{-5}	6.88×10^{-5}	0.654	0.515
Mean annual temperature:Mean UV radiation	-2.39×10^{-6}	4.68×10^{-6}	-0.512	0.610

5c

Molecular CP12 rate (log) ~ Body mass (log) + Hand-wing index + Mean annual temperature + Mean UV radiation

	Estimate	Standard error	t-value	p-value
(Intercept)	-0.429	0.288	-1.492	0.140
Body mass (log)	0.023	0.046	0.492	0.625
Hand-wing index	0.012	0.006	2.068	0.043
Mean annual temperature	-1.30×10^{-4}	0.005	-0.028	0.978
Mean UV radiation	1.14×10^{-5}	2.04×10^{-5}	0.557	0.579

6a

Molecular CP3 rate (square root) ~ Body mass (log) + Hand-wing index + Mean annual temperature + Mean UV radiation + logmass:Hand-wing index + Mean annual temperature:Mean UV radiation

	Estimate	Standard error	t-value	p-value
(Intercept)	2.021	1.499	1.348	0.183
Body mass (log)	0.270	0.372	0.726	0.471
Hand-wing index	0.059	0.071	0.835	0.407
Mean annual temperature	-0.042	0.058	-0.727	0.470
Mean UV radiation	-9.95×10^{-5}	1.55×10^{-4}	-0.642	0.523
Body mass (log):Hand-wing index	-0.014	0.021	-0.656	0.514
Mean annual temperature:Mean UV radiation	8.75×10^{-6}	1.06×10^{-5}	0.826	0.412

6b

Molecular CP3 rate (square root) ~ Body mass (log) + Hand-wing index + Mean annual temperature + Mean UV radiation + Mean annual temperature:Mean UV radiation

	Estimate	Standard error	t-value	p-value
(Intercept)	2.728	1.038	2.629	0.011
Body mass (log)	0.036	0.105	0.344	0.732
Hand-wing index	0.014	0.013	1.051	0.297
Mean annual temperature	-0.037	0.057	-0.641	0.524
Mean UV radiation	-9.37×10^{-5}	1.54×10^{-4}	-0.608	0.545
Mean annual temperature:Mean UV radiation	7.93×10^{-6}	1.05×10^{-5}	0.757	0.452

6c

Molecular CP3 rate (square root) ~ Body mass (log) + Hand-wing index + Mean annual temperature + Mean UV radiation

	Estimate	Standard error	t-value	p-value
(Intercept)	2.114	0.645	3.276	0.002
Body mass (log)	0.027	0.104	0.256	0.799
Hand-wing index	0.015	0.013	1.211	0.230
Mean annual temperature	0.006	0.010	0.565	0.574
Mean UV radiation	1.76×10^{-5}	4.59×10^{-5}	0.384	0.702

7a

CP12/CP3 ratio ~ Body mass (log) + Hand-wing index + Mean annual temperature + Mean UV radiation + logmass:Hand-wing index + Mean annual temperature:Mean UV radiation

	Estimate	Standard	t-value	p-value

		error		
(Intercept)	0.365	0.496	0.737	0.464
Body mass (log)	-0.144	0.123	-1.173	0.245
Hand-wing index	-0.025	0.023	-1.084	0.283
Mean annual temperature	0.033	0.019	1.703	0.094
Mean UV radiation	5.48×10^{-5}	5.13×10^{-5}	1.070	0.289
Body mass (log):Hand-wing index	0.007	0.007	1.014	0.314
Mean annual temperature:Mean UV radiation	-6.4×10^{-5}	3.5×10^{-5}	-1.826	0.073

7b

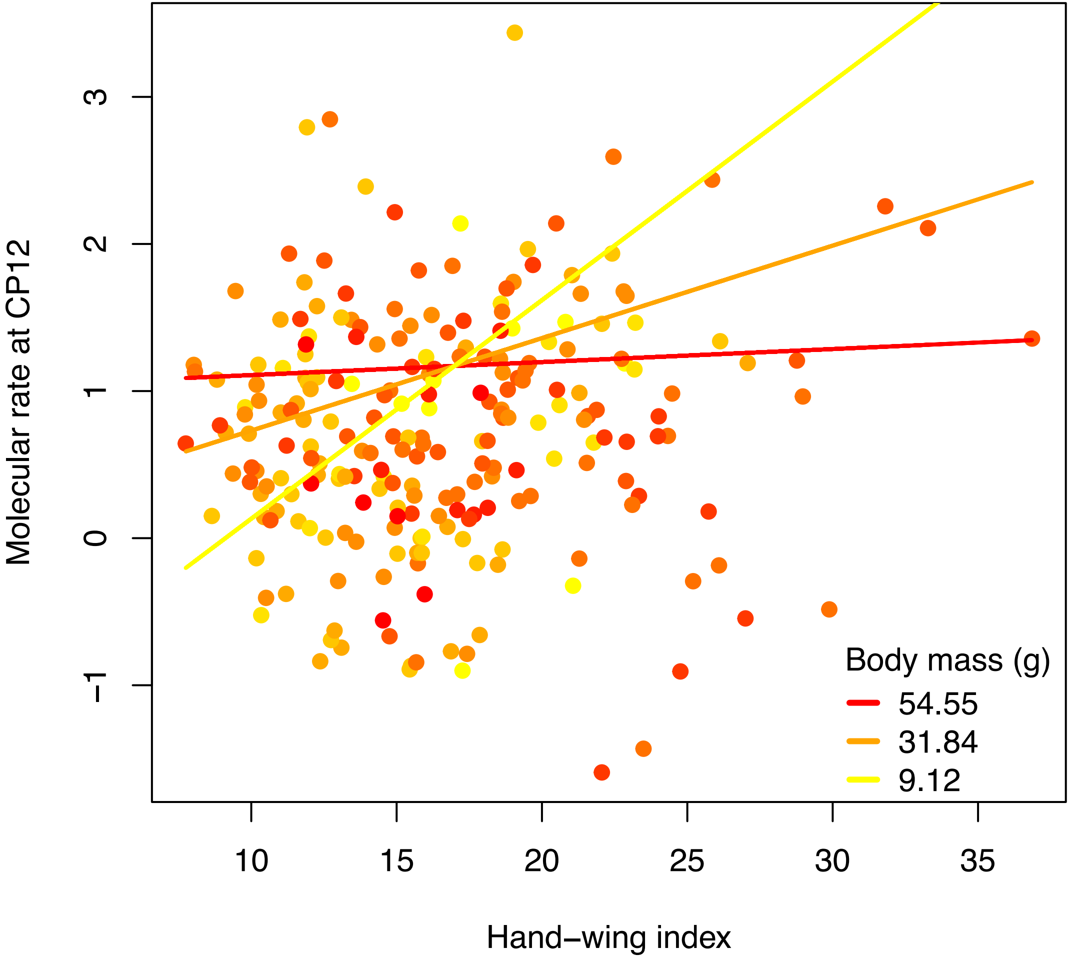
CP12/CP3 ratio ~ Body mass (log) + Hand-wing index + Mean annual temperature + Mean UV radiation + Mean annual temperature:Mean UV radiation

	Estimate	Standard error	t-value	p-value
(Intercept)	0.004	0.345	0.012	0.991
Body mass (log)	-0.025	0.035	-0.710	0.480
Hand-wing index	-0.002	0.004	-0.476	0.635
Mean annual temperature	0.030	0.019	1.572	0.121
Mean UV radiation	5.18×10^{-5}	5.12×10^{-5}	1.013	0.315
Mean annual temperature:Mean UV radiation	-5.97×10^{-6}	3.48×10^{-6}	-1.717	0.091

7c

CP12/CP3 ratio ~ Body mass (log) + Hand-wing index + Mean annual temperature + Mean UV radiation

	Estimate	Standard error	t-value	p-value
(Intercept)	0.466	0.218	2.137	0.036
Body mass (log)	-0.018	0.035	-0.501	0.618
Hand-wing index	-0.003	0.004	-0.788	0.434
Mean annual temperature	-0.002	0.004	-0.631	0.530
Mean UV radiation	-3.2×10^{-5}	1.55×10^{-5}	-2.064	0.043


Molecular rate at CP12
−1
0
1
2
3
10
15
20
25
30
35
Hand−wing index
Body mass (g)
54.55
31.84
9.12