1   **Molecular analyses reveal consistent food web structure with**

2   **elevation in rainforest *Drosophila* - parasitoid communities**

3   **Authors:**

4   [1]Christopher T. Jeffs, (Joint first)

5   [1,2] J. Christopher D. Terry (Joint first) 0000-0002-0626-9938

6   [3]Megan Higgie 0000-0002-2397-0240

7   [4]Anna Jandová

8   [4]Hana Konvičková

9   [4,5]Joel J. Brown 0000-0002-3608-6745

10   [4]Chia Hua Lue

11   [6]Michele Schiffer 0000-0002-5203-2480

12   [7]Eleanor K. O'Brien 0000-0001-5145-7340

13   [7,8]Jon Bridle

14   [4]Jan Hrcek (co-last) 0000-0003-0711-6447

15   [1]Owen T. Lewis (co-last) 0000-0001-7935-6111

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17   **Affiliations:**

18   1, Department of Zoology, University of Oxford, Oxford, UK,

19   2, School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London E1

20   4NS, UK

21   3, College of Science and Engineering, James Cook University, Townsville QLD 4811, Australia

22   4, Biology Centre, Czech Academy of Sciences, Institute of Entomology, Ceske Budejovice, Czech Republic

23   5, University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic

24   6, Daintree Rainforest Observatory, James Cook University, Cape Tribulation, Australia.

25   7, School of Biological Sciences, University of Bristol, Bristol, BS8 1UD UK

26   8, Department of Genetics, Environment and Evolution, University College London, London, UK,

27   Abstract

28   The analysis of interaction networks across spatial environmental gradients is a powerful approach

29   to investigate the responses of communities to global change. Using a combination of DNA

30   metabarcoding and traditional molecular methods we built bipartite *Drosophila*-parasitoid food

31   webs from six Australian rainforest sites across gradients spanning 850 m in elevation and 5° Celsius

32   in mean temperature. Our cost-effective hierarchical approach to network reconstruction separated

33   the determination of host frequencies from the detection and quantification of interactions. The

34   food webs comprised 5-9 host and 5-11 parasitoid species at each site, and showed a lower

35   incidence of parasitism at high elevation. Despite considerable turnover in the relative abundance of

36   host *Drosophila* species, and contrary to some previous results, fundamental metrics of network

37   structure including nestedness and specialisation did not change significantly with elevation.

38   Advances in community ecology depend on data from a combination of methodological approaches.

39   It is therefore especially valuable to develop model study systems for sets of closely-interacting

40   species that are diverse enough to be representative, yet still amenable to field and laboratory

41   experiments.

42   Keywords

43   DNA metabarcoding, elevational gradients, Drosophila, host-parasitoid, quantitative network

44   Introduction

45   The geographic distribution of a species is determined by its intrinsic abiotic limits and by its
46   interactions with other species. Individual populations of species do not respond to environmental
47   changes in isolation from the species with which they interact (Davis et al. 1998, Gilman et al. 2010,
48   Urban et al. 2016). Furthermore, interspecific interactions themselves respond to environmental
49   change in diverse ways (Tylianakis et al. 2008, Thierry et al. 2019, Guiden et al. 2019). To understand
50   how species' distributions will respond to global change, it is therefore important to consider this
51   wider community context.

52   One valuable approach for understanding how ecological communities will respond to
53   environmental change is to study how food webs and other networks of interspecific interactions
54   vary along elevational gradients (Tylianakis and Morris 2017, Pellissier et al. 2018, Baiser et al. 2019,
55   Gravel et al. 2019). Temperature changes rapidly with elevation, so transects spanning elevations on
56   mountain slopes can approximate aspects of climate change, while avoiding many of the
57   confounding factors that complicate analyses of latitudinal gradients (Hodkinson 2005, Körner 2007,
58   Malhi et al. 2010, Morris et al. 2015). There is an expectation that predation and parasitism risk
59   increase with temperature (Roslin et al. 2017, Libra et al. 2019), but it is unclear how such changes
60   are expressed at the community level. Quantitative food webs should be particularly valuable in
61   exploring changes in species interactions with changes in temperature, because they incorporate the
62   relative abundances and interaction frequencies of component species (Memmott and Godfray
63   1994). The structure of communities can be summarised with network-level metrics that can reveal
64   shifts that are not apparent in simple connectance food webs or in qualitative descriptors of
65   diversity such as species richness (Tylianakis et al. 2007, Delmas et al. 2019).

66   To date, there have been relatively few network analyses along elevational transects (Pellissier et al.
67   2018), in part because of the substantial research effort required to document them through direct
68   observation (for example, by observing herbivores feeding on plants, or parasites associated with
69   hosts). These traditional approaches to describing networks are highly labour-intensive, even for a
70   single network. Rapid, accurate and cost-effective methods to characterise interaction networks are
71   therefore needed to assess the community-wide consequences of environmental change.

72   Recent developments in molecular methods offer the potential to document interaction networks
73   with lower 'per-interaction' effort, while also addressing biases and deficiencies such as those arising
74   from insufficient sampling. Traditional methods of food web construction are also often prone to
75   sampling biases (Gibson et al. 2011) and can miss cryptic species and associated interactions
76   (Derocles et al. 2015). By contrast, DNA-based approaches can be faster, more efficient and
77   taxonomically more comprehensive, allowing the simultaneous resolution of interactions and
78   identification of morphologically-cryptic species (Hrček and Godfray 2015). Molecular approaches to
79   the construction of ecological networks have become increasingly common over the last decade
80   (Clare 2014, Evans et al. 2016, Derocles et al. 2018, Roslin et al. 2019, Evans and Kitson 2020).
81   Although high throughput, 'next generation sequencing' (NGS) methods are now routinely used to
82   characterise bulk samples, their use for characterising trophic interactions in food webs has proved

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83   more challenging. Recently-developed nested DNA metabarcoding allows large numbers of pooled
84   samples to be processed in a single NGS run without losing resolution to individual samples (Kitson
85   et al. 2019). This method has been used successfully to identify interactions involving a single species
86   and its consumers (Kitson et al. 2019). However, it is unclear whether such success is scalable to
87   more complex interaction networks.

88   Food webs involving insect parasitoids, whose larvae feed exclusively on, and kill, a single arthropod
89   host (Godfray 1994), have considerable potential for studying the processes that structure
90   communities as they are both experimentally tractable while retaining meaningful levels of
91   complexity (Hrček and Godfray 2015). Host-parasitoid interactions are amenable to molecular
92   identification (Hrček et al. 2011, Wirta et al. 2014, Derocles et al. 2015, Kitson et al. 2019) and are
93   important drivers of insect population dynamics (Hassell 2000). Climate change is expected to cause
94   distribution shifts in parasitoids (Burrows et al. 2014), alter host-location and utilisation, and change
95   the balance between parasitoid virulence and the immune response of their hosts (Hance et al.
96   2007). The consequences for host-parasitoid communities and the functions and services that they
97   mediate are likely to be pronounced (Jeffs and Lewis 2013, Derocles et al. 2018, Thierry et al. 2019).
98   Previous work has found increased specialism of host-parasitoid networks with increasing elevation
99   (Maunsell et al. 2015). However, other sources of information about the effect of temperature, such
100   as latitudinal trends, do not show a consistent trend in specialism or other community-level
101   properties (Morris et al. 2014, Moles and Ollerton 2016). Meta-analyses have found scale
102   dependence of patterns (Galiana et al. 2019), but there is a need for additional studies to assess
103   general trends in network structure.

104   Here we explore how food webs might respond to predicted climate change by describing the
105   community of wild frugivorous vinegar flies (Diptera: Drosophilidae) and their parasitoids across two
106   elevational transects in Australian rainforest. Biotic interactions (O'Brien et al. 2017, 2018) and
107   thermal tolerance (Kellermann et al. 2012) have been shown to determine *Drosophila* sp. ranges. In
108   this study, we resolve and quantify food web composition and structure using molecular methods.
109   We demonstrate how nested DNA metabarcoding can be used to generate multiple quantitative
110   networks across landscapes, opening up opportunities to use this and similar systems as models in
111   community ecology research.

Methods

Study sites

114   The Australian Wet Tropics World Heritage Area is an area of rainforest with exceptional levels of
115   biodiversity (Stork et al. 2011) which lies close to Queensland's northeast coast between Cooktown
116   and Townsville (15-19'S, 145-146.30'E). We established study sites along two transects through the
117   rainforest along elevation gradients at Paluma Range Road in Paluma Range National Park and
118   Kirrama Range Road within Girramay National Park (Figure 1). Our study sites spanned elevations
119   from 59 – 916 m above sea level and mean temperatures ranged from 21°C at the higher elevation
120   sites to 26 °C at lower sites (SI 1). Variability in temperature was not strongly related to elevation
121   over this period, although the lower elevations show higher variation annually. The temperature
122   differences across our elevation transects are broadly consistent with temperature shifts expected
123   over the next 50-100 years under models of climate change (IPCC 2014). We selected seven forested
124   sites along each transect spanning the three 'ecologically significant climatic zones' identified by

125   Webb and Tracey (1981) on the basis of changes in forest structure with elevation.

Study system

127   We focused on the food web of interactions involving native, forest-dwelling, fruit-feeding

128   *Drosophila* species and their associated parasitoids (Hymenoptera). Well-established laboratory

129   practices and fundamental knowledge of *Drosophila* and their parasitoids (Kraaijeveld and Godfray

130   1997, Markow and O'Grady 2008, Prevost 2009) make these interacting species a valuable model

131   system in community ecology. In our study area, *Drosophila* assemblages associated with rotting

132   fruit are well characterised (Hangartner et al. 2015), although less is known about their parasitoids.
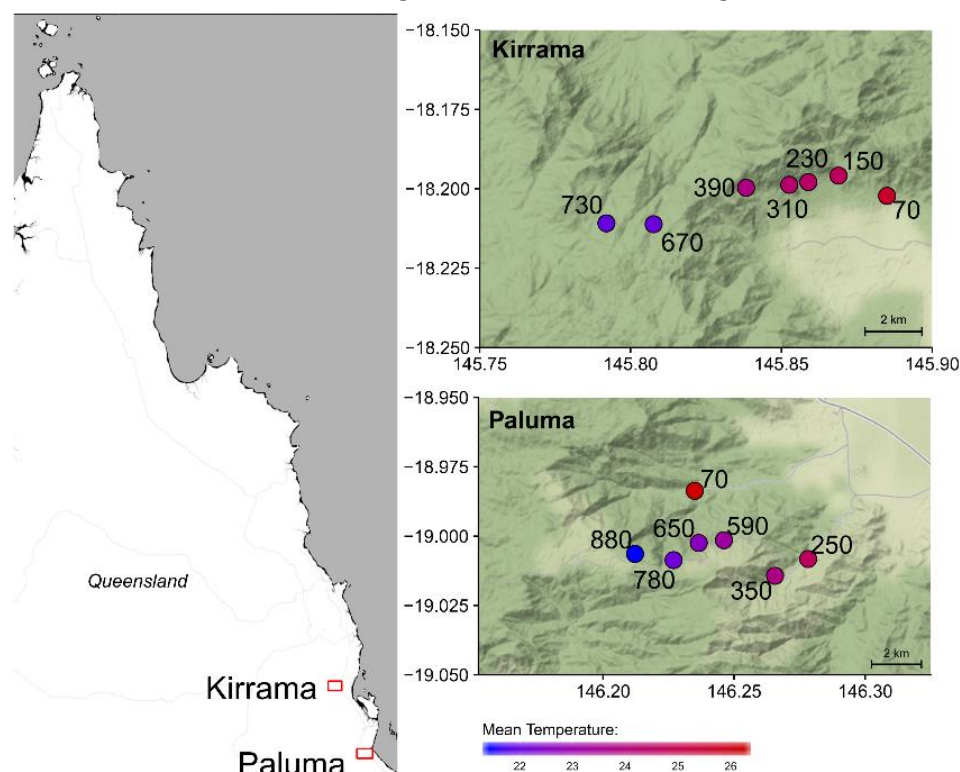

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Figure 1. Location of transects in north-eastern Australia. Location of sites are coloured by the mean
135   temperature recorded by dataloggers at each site during the research period, and labelled by the elevation
136   (metres above sea level). Map tiles by Stamen Design, under CC BY 3.0. Data by OpenStreetMap, under ODbL.

Sampling Method

138   At each site, *Drosophila*-parasitoid communities were sampled using bottle traps baited with

139   fermented banana. Bottle traps consisted of 1.5 L plastic water bottles with two 8cm x 10cm

140   windows cut in the side to allow flies and wasps to enter, following Markow and O'Grady (2006). A

141   rain-shield was placed over the lid of the bottle and the line was coated with Tanglefoot® insect

142   barrier (The Tanglefoot Company, Grand Rapids, USA) to prevent ants compromising the traps (see

143   image in SI 1). Bottle traps were hung 1.5 m above the ground and separated by a minimum of 5 m.

144   We set the traps in shaded locations at least 5 m from a roadside edge or canopy gap.

145   Previous work has established that banana bait samples the native generalist *Drosophila* spp. in our

146   focal communities effectively (Schiffer *pers. obs.*, Carson and Okada 1983). Traps were baited with

147   50 g of banana bait (mashed 'Cavendish' bananas supplemented with bakers' yeast), and strips of

148   cardboard were added to facilitate *Drosophila* pupation. Traps were exposed during the period 11th

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149   March – 12th April 2016, corresponding to mid-late wet season when insect activity is high.
150   Parasitoids of *Drosophila* attack either larvae or pupae, but all emerge from pupae. Thus, by
151   sampling *Drosophila* pupae we were able to document the network of host-parasitoid trophic
152   interactions because pupae contain information about both the host and any parasitoid infecting it.

153   To capture the variation in colonisation speed and pupation time of different *Drosophila* species and
154   across the elevation gradient, traps were open for either long (24 days), medium (14-15 days), or
155   short (11-12 days) exposure periods at all sites. We did not find evidence of exhaustion of baits,
156   indicating that competition for food was unlikely to consistently exclude components of the
157   community.

158   For each of the sites we sorted fly pupae from 15 randomly selected trap samples, five from each of
159   the three exposure-time categories. We excluded traps if they had been compromised by ants or
160   mammals, were exceptionally dry, or had been flooded by rain. Traps were either sorted on the day
161   of collection or frozen at -15°C and sorted later. Pupae were stored individually in separate wells of
162   96-well plates filled with 96% ethanol and stored at -15°C. A random subset of pupae from the high
163   and low elevation sites were reared in sealed, ventilated 96-well plates in a 22°C incubator to yield
164   adult fly and parasitoid material. These were identified morphologically (*Drosophila* to species level
165   by MS and parasitoid to genus level by C-HL) and used to build a DNA reference library (Table S2.1).

Hierarchical Network Construction Approach

167   Quantitative interaction networks were built for three sites from both transects representing the
168   highest, lowest and most central elevations. Samples were taken from all three exposure times in
169   proportion to the number caught in each. Quantitative networks were generated by a hierarchical
170   sampling strategy. First, a 'core' network was built from a random selection of 182 pupae per site,
171   where both host and parasitoid (if present) were identified by molecular methods (detailed below).
172   In this stage of network construction, 722 hosts were successfully identified in 86-138 samples per
173   site. Of these, a total of 179 were parasitised, and in 147 cases the parasitoids were successfully
174   identified.

175   Next, to optimise the number of interactions sampled from each network, all available additional
176   samples (total 2659, 339-564 per site) were screened for parasitoid DNA using PCR. Those found to
177   be parasitized were then sequenced to identify the host and parasitoid. This resulted in an additional
178   289 identified interactions (15-119 per network). These two sets of data were combined into an
179   overall 'enriched' quantitative network using relative host frequencies and overall host-specific
180   parasitism rates from each 'core' network, but specific interaction frequencies derived from both the
181   core and enriching sets. In a few cases a host was identified in the 'enriching' interaction set, but not
182   found in that site's 'core' network. These host species were assigned a relative abundance of 0 in the
183   enriched network. However, these interactions were included in the specification of the site-specific
184   binary interaction matrices used for the calculation of site dissimilarity and qualitative network
185   metrics.

Molecular methods to describe network structure

187   We used a combination of DNA metabarcoding and classic molecular methods. We first performed
188   DNA metabarcoding on a selection of individually tagged pupal samples ("core" samples). By
189   comparing the sequences to a custom reference library, we were able to identify hosts and estimate

190   overall host diversity. Contrary to expectations based on previous work (Kitson et al. 2019),
191   metabarcoding did not allow parasitoid identification and therefore we could not use this approach
192   to document and quantify host-parasitoid interactions. We therefore developed a number of classic
193   molecular tools to obtain this missing information and allow cost-effective identification of
194   thousands of samples. The classic tools were: i) multiplex PCR to identify the host species, followed
195   by ii) Sanger sequencing of samples not identified by the multiplex, and iii) parasitoid detection and
196   identification using PCR and Sanger sequencing. Full details are given in Supplementary Information
197   2.

Patterns in *Drosophila* occurrence and parasitism rate

199   To measure rates of parasitism, we supplemented the data described above with additional
200   screening results from 350 samples from each of the remaining four sampling sites along each
201   transect, generating a grand total of 6780 parasitism assays. To explore trends in parasitism we fit
202   minimal generalised linear models (GLMs) with a quasi-binomial error distribution, using elevation
203   (metres a.s.l.) and transect (Kirrama or Paluma) as predictors. The significance of individual terms
204   was assessed using F-tests. Since host-species turnover may mask elevation effects if species have
205   different susceptibilities, we also fitted the model including *Drosophila* species as an additional
206   categorical predictor. This analysis was restricted to three abundant *Drosophila* species observed at
207   all six core sites (*D. pallidifrons, D. pseudoananassae* and *D. rubida*).

Network metrics across the elevation gradient

209   We calculated five standard network statistics for each site, using the *bipartite* R package (Dormann
210   et al. 2008): *H2'*, which measures overall level of specialisation in the network (Blüthgen et al. 2006);
211   *weighted NODF*, a metric of nestedness that corrects for network size and connectance (Almeida-
212   Neto et al. 2008); *quantitative modularity*, which assesses the extent to which the network is divided
213   into discrete compartments (Beckett 2016); *network vulnerability*, the mean number of parasitoid
214   species attacking each host; and *network generality*, the mean number of host species that each
215   parasitoid attacks. We tested for the impact of elevation on each metric with a linear model,
216   including the matrix size (number of hosts × number of parasitoids, following Morris et al. (2014)
217   and transect (Kirrama or Paluma) as additional predictors.

218   To assess the precision with which we can infer these metrics given our sample sizes, we used
219   bootstrapping of our available observations. For the enriched networks, we resampled each
220   sampling stage separately. Although bootstrapping networks can provide an indication of the degree
221   of uncertainty in a metric value, because the networks can only become smaller the approach can
222   introduce biases.

Drivers of interaction turnover

224   Interaction β-diversity represents differences in the set of observed interactions between sites
225   (Poisot et al. 2012, Graham and Weinstein 2018). Whole-network dissimilarity (β_{WN}) between two
226   sites can be partitioned into that attributable to species not being present at both sites (β_{ST}) and
227   that attributable to interactions only occurring at one site despite both species being present at both
228   (β_{OS}). Because methods partitioning quantitative similarity measures are not yet well established,
229   we used the qualitative Jaccard similarity metric (Novotny 2009) and a customised version of the

230   *betalinkr* function from the *bipartite* R package that takes into account host species observed at a
231   site that were not observed interacting with parasitoids. We enforced $\beta_{WN} = \beta_{ST} + \beta_{OS}$ by dividing
232   β_{ST} and β_{OS} through by $\beta_{WN}/(\beta_{ST} + \beta_{OS})$ following Poisot et al. (2012). For additional comparison,
233   we calculated the Jaccard similarity between the host species found at each site (β_{Host}). For each of
234   these metrics, we calculated pairwise dissimilarities between each our six core sites. We used
235   multiple regression on distance matrices (Legendre et al. 1994) via the *ecodist* R package (Goslee
236   and Urban 2007) to investigate whether these dissimilarities are driven by elevational or between-
237   transect differences.

Results

Parasitism Rate

240   Overall parasitism rate declined significantly with elevation. For each 100 m rise in elevation, the
241   probability of being parasitized decreased by 14% (coefficient estimate $\beta_{elevation}$= -0.0015 m^{-1}, se =
242   0.0005, Pr(>F) = 0.00865). Parasitism was significantly higher overall at Paluma than at Kirrama
243   ($\beta_{Paluma} = 1.06$, Pr(>F)= 0.0023). The elevational trend was largely driven by data from the Paluma
244   transect (Figure 2a), although the interaction term between elevation and transect was not
245   statistically significant (Pr(>F) = 0.135). However, the high over-dispersion in the data (the dispersion
246   parameter was estimated to be 14.8) makes it challenging to identify interaction terms with
247   confidence.

248

249   Parasitism rates of widespread *Drosophila* species showed similar patterns (Figure 2b). Model
250   selection did not identify any host-specific transect (Pr(>F) = 0.90) or elevation (Pr(>F) =0.21) effects
251   but did find a significantly greater decline in parasitism rate with elevation at Paluma (Pr(>F) = 0.035
252   0.016). At Kirrama, there was only a negligible effect of elevation ($\beta_{elevation_K}$= -0.00016 m^{-1}, se:
253   0.00085). Full statistical results and model selection procedures are given in SI 3.
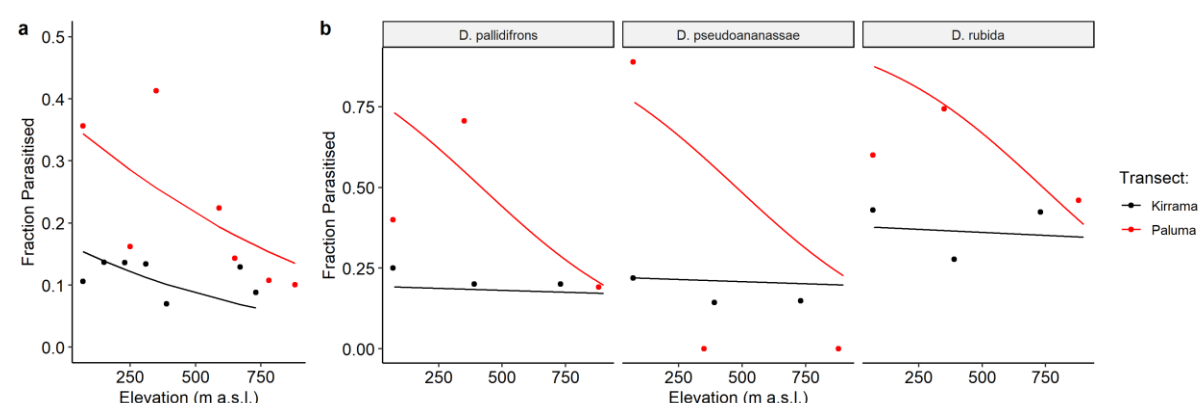
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Figure 2. a) The fraction of pupae from traps at each site observed to be parasitized in relation to elevation. b) Parasitism rates at the six core sites for the three most abundant species in relation to elevation. In both subfigures, lines show the fitted minimal models.

7

Network Structure

260 Across all sites we found 12 *Drosophila* species, 15 parasitoid species (principally Figitidae, Diapriidae
261 and Braconidae, Table S2.3), together making up 55 different interaction pairs. The host species
262 found at Kirrama were a subset of those observed at Paluma. The six enriched quantitative networks
263 are shown in Figure 3. There was no significant relationship between any of the tested network
264 metrics and elevation (p>0.05 in all cases, Figure 4 & SI 3). Including network size as an additional co-
265 predictor did not change this. Support intervals from resampling are shown in Figure 4.

266
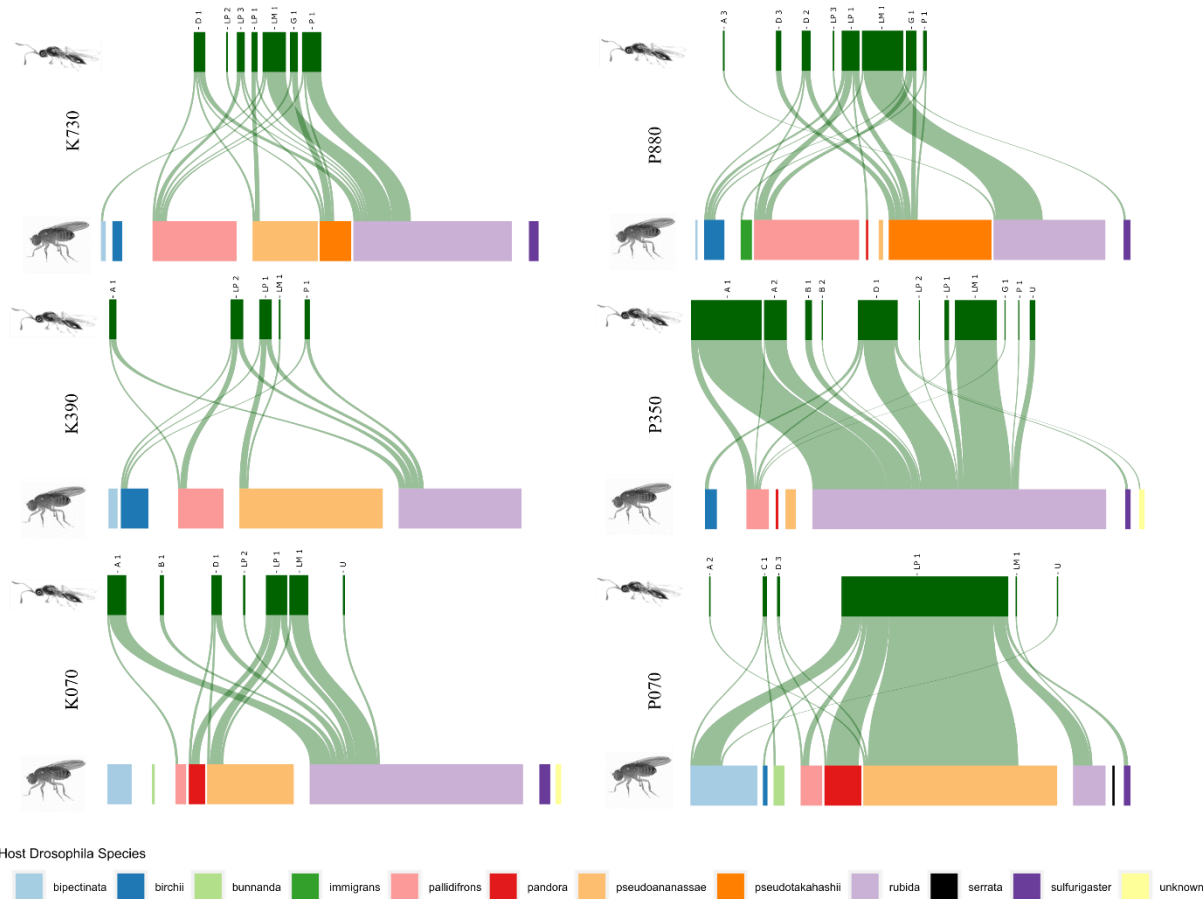
Figure 3. Quantitative interaction networks from each site, calculated using the enriching method described in
the main text. The width of the boxes corresponds to the relative frequency that each species or interaction
was observed. Networks are arranged in elevation order, with Kirrama on the left and Paluma on the right and
labelled by the elevation of the site. Networks are drawn using the *bipartiteD3* R package. Parasitoid wasps are
labelled by taxon: A= *Asobara*, B = Braconidae, C = Chalcidoidea, D= Diapriidae, G= *Ganapsis*, LM =
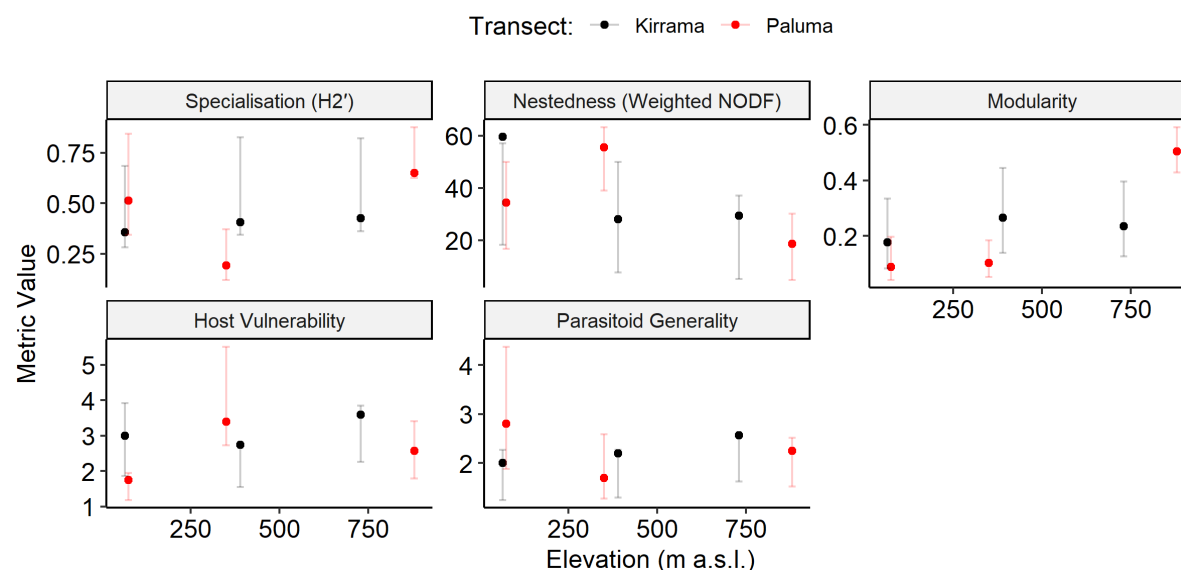*Leptolamina*, LP = *Leptopilina*, P = Pteromalidae, U = Unidentified.

Figure 4. Consistency in network-level metrics across the elevational transect, with no statistically significant effect of elevation in all cases. Error bars show bootstrapped 95% support intervals from resampling the observations at each site.

Drivers of network dissimilarity

Species turnover (β_{ST}) was a more important driver of qualitative dissimilarity between networks (β_{WN}) than changes in observed interactions (β_{OS}, Figure 5a). Although dissimilarity tended to be higher for pairs of networks from locations differing greatly in elevation (Figure 5a), neither transect differences nor elevational differences significantly explained any of the components of network dissimilarity (all unadjusted p > 0.1). Overall host community composition disimilarity (β_{Host}) was not significantly related to elevational differences (p = 0.45) or transect differences (p = 0.78).
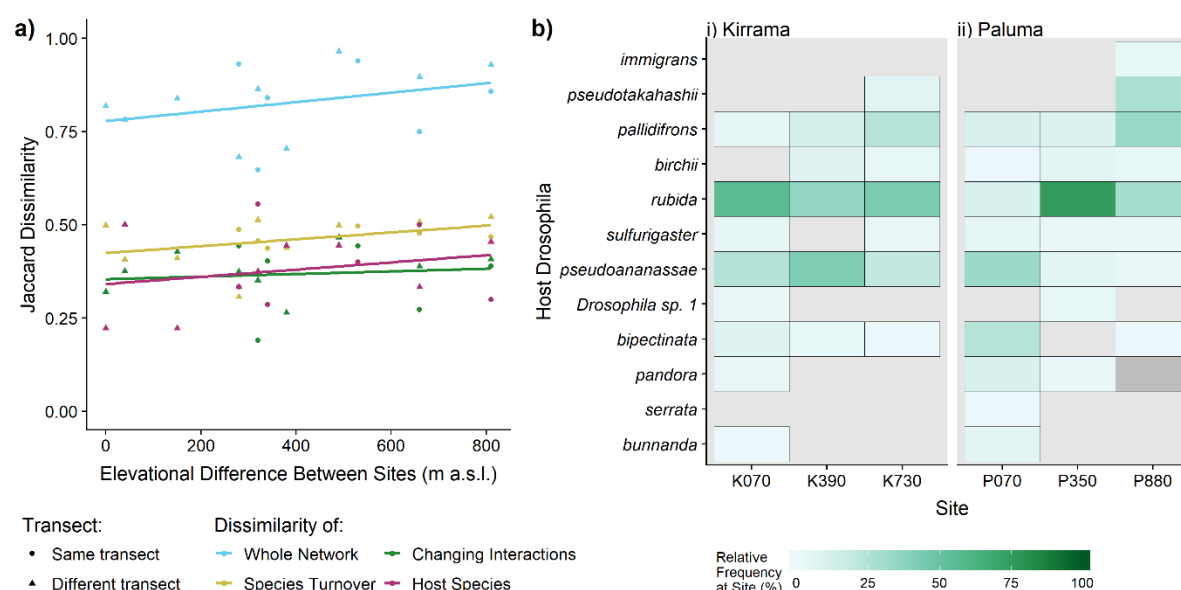

Figure 5. a) Effect of elevational difference on between-site community β-diversity measures. Whole-network interaction dissimilarity (β_{WN}) was large, and predominantly composed of species turnover (β_{ST}), although there was also a notable contribution derived from changing interactions between present species (β_{OS}).

289   Overall Jaccard dissimilarity in host species composition (β_{Host}) was also notable. Although all the dissimilarity
290   measures show an apparent increase with elevational difference, these trends were not significant. Lines show
291   a direct linear model through each dissimilarity metric, ignoring transect differences. **b)** Relative observation
292   frequency of the host species observed at each site. Host species are arranged in order of the centre of mass
293   of their relative abundances.

Discussion

295   Our study demonstrates the capacity of molecular methods to sample multiple interaction networks
296   of a model system efficiently and to shed light on the underlying ecological processes. We found
297   evidence for a reduction in parasitism frequency at higher elevations in line with previous results
298   from comparable systems (Kimura and Suwito 2015), although this was largely driven by one of our
299   two transects. Despite this, we found no evidence for changes to food web network structure along
300   an elevation gradient that spanned a temperature range comparable to the ecological range of many
301   of the focal species. This contrasts with the results from previous studies of host parasitoid food
302   webs of Maunsell et al. (2015) and Morris et al. (2015) and suggests that any shifts in network
303   structure with elevation are unlikely to be consistent and that, by extension, the response of host-
304   parasitoid networks to climate change may not be easily predicted (Hance et al. 2007).

305   The lack of significant trends in network structure with elevation are consistent with the absence of
306   marked trends in these metrics across latitudes documented by Morris et al. (2014) and the
307   apparent constancy across landscapes in more focussed studies of antagonistic interaction networks
308   (Kemp et al. 2017, Redmond et al. 2019). The sample size for each of our networks is still rather
309   small, with a number of interactions represented by only a single observation. This makes it likely
310   that many interactions remain unobserved (Jordano 2016). Nonetheless, the quantitative network
311   metrics we used are frequently found to be amongst the most robust to moderate levels of sampling
312   (Banašek-Richter et al. 2004, Fründ et al. 2016, Vizentin-Bugoni et al. 2016, Henriksen et al. 2019).

Identifying drivers of community change

314   We found a high degree of network dissimilarity between sites at both Kirrama and Paluma, which
315   derived largely from species turnover (both host and parasitoid) rather than changes in the observed
316   interactions. Although the transects have similar temperature profiles, the vegetation structure on
317   the Kirrama transect had been more heavily disrupted by a cyclone five years before the sampling
318   period. However, we did not identify any significant differences between the transects. We did not
319   identify any significant effect of elevational differences on community differences, as measured by
320   binary Jaccard dissimilarity. This pattern of higher-level consistency mirrors that found in a number
321   of systems (Kaartinen and Roslin 2012, Kemp et al. 2017, Gravel et al. 2019, Redmond et al. 2019)
322   and suggests that there are mechanisms channelling communities towards particular structures,
323   despite external changes.

324   Although there are known shifts in relative abundance of host species in our study system with
325   elevation (for example, *D. birchii* has higher abundance at cool, high-elevation sites than in warmer,
326   low-elevation sites (O'Brien et al. 2017)), many of the host species occur across the full range of
327   elevations sampled, leaving only a minority of high or low elevation specialists to drive any
328   qualitative differences (Figure 5b). In terms of the interactions themselves, we found relatively high
329   levels of generality – the mean level of specificity (as measured by *H2'*) in our communities (0.42)

330 was notably lower than the mean of 0.65 found in a cross-latitude survey of host-parasitoid
331 networks (Morris et al. 2014). Although the majority of species were found across the elevation
332 range, their relative abundances varied considerably with elevation (Figure 5b). Differences in
333 network composition may be difficult to detect with the binary network dissimilarity methods we
334 used, and emphasise changes in comparatively rare species, which are more likely to be influenced
335 by sampling effects (Chao et al. 2005). This highlights the importance of recording abundance rather
336 than spatial range limits in determining species' responses to temperature.

337 To address this challenge, Staniczenko et al. (2017) proposed that changes in relative interaction
338 frequency could be used to link quantitative changes in interaction frequencies to environmental
339 changes. We attempted to use such an approach within a Bayesian framework to relate elevation to
340 per-capita interaction frequencies (*not shown*). However, we could not confidently identify any
341 strong evidence for individual interactions being modified across the gradient, even when restricting
342 analysis to the most widely observed interactions. Without independent external estimates of the
343 relative abundance of parasitoid species, analysis of changing interaction rate is severely restricted.
344 More fundamentally, variation in the identity and strength of links within networks along
345 environmental gradients may be driven by any combination of turnover in species composition,
346 changes in species abundances and by abiotic influences on species interactions (Pellissier et al.
347 2018). Moving from 'how' to 'why' is a critical challenge in network ecology, but feedbacks between
348 the relative abundance of each species and the interactions between them generate fundamental
349 circularities (Dormann et al. 2017, Moreira et al. 2018). An essential part of future work to identify
350 the drivers of community composition will be controlled manipulative experiments (Derocles et al.
351 2018). This and other *Drosophila*-parasitoid systems offer exceptional potential for field
352 manipulations (O'Brien et al. 2017, 2018), identification of physiological traits (Kellermann et al.
353 2012) and laboratory experiments (Davis et al. 1998).

Sampling strategy and molecular methods

355 Despite the per-sample cost of molecular methods falling significantly in recent years, identification
356 effort is still a finite and valuable resource. Our hierarchical sequencing approach was helpful in
357 increasing the number of identified interactions in our dataset for a given amount of laboratory
358 effort, an approach that can help to maximise scarce resources and particularly relevant in species-
359 rich interaction networks. One drawback is that the additional data wrangling required to construct
360 quantitative networks from the resulting data can complicate subsequent analyses, and in particular
361 to propagate estimates of uncertainty in network structure.

362 Our sampling strategy was only a single snapshot of the interaction network at each of our sites, and
363 therefore does not resolve seasonal or annual turnover in interactions (Kemp et al. 2017, Lue et al.
364 2018). Our approach of gathering pupae samples after a variety of exposure times enables us to
365 characterise a community where development time varies among species and with temperature.
366 However, a number of challenges remain. For example, shorter exposure periods are likely to
367 underestimate the abundance of host species with longer development times. Species with rapid
368 development times may therefore be over-represented in our sampling, although we did not detect
369 consistent differences in the host composition between our exposure periods. Furthermore, rather
370 than parasitism rate, our interactions are more accurately described as attack-rates, since it is not
371 necessarily the case that a parasitoid egg or larva within a host would develop successfully, kill the

372   host and emerge as an adult (Condon et al. 2014). Rearing to emergence is the only way to establish
373   the result of a parasitism event definitively (Hrček and Godfray 2015), but this is much more labour
374   intensive, introduces other biases (such as survival in laboratory conditions), and would negate the
375   advantages of the molecular approach.

376

377   DNA metabarcoding alone was unable to describe our host-parasitoid networks without
378   supplementary use of conventional molecular methods. Although Kitson et al. (2019) demonstrated
379   the DNA metabarcoding method on a single host and parasitoid species, we were unable to obtain
380   network data with this method alone in our multispecies system. We estimate that increasing
381   sequencing coverage per sample by several orders of magnitude would be necessary to characterise
382   parasitoids and their links to hosts reliably. This would not currently be cost-effective, but might
383   become more realistic in the near future as sequencing costs fall. We recommend using a single-step
384   PCR with larger number of primer tags in future studies, since the nested metabarcoding approach
385   involving two PCRs may be more prone to mistagging. We used a strategy for detecting mistagging
386   developed by Kitson et al (2019) and found it to be a crucial aspect for producing reliable and
387   verifiable DNA metabarcoding data. We therefore recommend incorporating mistagging controls to
388   DNA metabarcoding runs in addition to negative and positive controls.

389   Conclusion

390   We documented pronounced turnover of *Drosophila* species relative abundance across an elevation
391   and temperature gradient. A strong reduction in parasitism with elevation was not associated with a
392   detectable change in overall network structure. DNA metabarcoding proved useful to characterise
393   these previously unstudied ecological networks, but it did not prove possible to use this approach
394   "out of the box" to obtain network data. Rather, metabarcoding data had to be supplemented with
395   data from classic molecular methods and a reliable DNA reference library. Ecological communities
396   are highly complex and communities will have to respond to multiple changes under global change.
397   To understand the patterns in these networks and to distinguish the principal drivers behind them,
398   manipulative experiments in the field and laboratory will be needed. A focus on tractable model
399   systems will allow an in-depth investigation into fundamental ecological dynamics of natural
400   systems.

401   **Author Contributions:**
402   CTJ: Led fieldwork, drafting manuscript, sampling design, performed molecular work. JCDT: Led statistical
403   analysis and writing of manuscript. MH: Contributed to funding application, supervised sampling and
404   fieldwork. AJ: Contributed to development of molecular methods, performed molecular work, HK: Contributed
405   to development of molecular methods, performed molecular work. JJB: Performed molecular work. CHL:
406   Parasitoid taxonomy, built parasitoid reference library, MS: *Drosophila* taxonomy. EO-B: Contributed to
407   funding application, sampling design and fieldwork. JB: Conceived project, secured funding, contributed to
408   sampling design. JH: Secured funding, led molecular work, writing. OTL: Conceived project, secured funding,
409   study design, writing. All authors contributed to editing of the final manuscript.
410

421

422 **Code and Data availability:**
423 Code to replicate all analyses is available on Github at
424 https://github.com/jcdterry/AusDrosTransect_CodeandData

425

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