Capture mechanism in Palaeotropical pitcher plants (Nepenthaceae) is constrained by climate

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Received: 6 May 2013 Returned for revision: 3 June 2013 Accepted: 3 July 2013

INTRODUCTION

The monotypic family Nepenthaceae comprises approx. 120 species of carnivorous pitcher plants (McPherson, 2009). Invertebrate prey is attracted to the pitcher via a combination of nectar, colour patterns and, in some cases, fragrance (e.g. Moran, 1996; Moran et al., 1999, 2012; DiGiusto et al., 2008, 2010). Nepenthes benefit from prey-derived nutrients, which augment the supply available via the roots (Moran and Moran, 1998; Pavlović et al., 2007). The centre of diversity of Nepenthes comprises the islands of Borneo, Sumatra, Sulawesi and the Philippines; the range extends north to Assam, west to Madagascar and south-east to Australia and New Caledonia (Danser, 1928). Nepenthes pitcher production incurs significant resource costs (Osunkoya et al., 2007, 2008). Therefore, in common with other carnivorous plants, Nepenthes pitcher plants are restricted to habitats in which the nutritional benefits from carnivory outweigh the costs of producing carnivorous organs (Givnish et al., 1984). As a consequence, members of the genus are somewhat patchily distributed across their range. For example, they are not typically found in the lowland dipterocarp forests that historically covered much of the region. It has been suggested that the Nepenthaceae provide an example of adaptive radiation, based on pitcher specialization for nutrient capture (e.g. Chin et al., 2010; Pavlović, 2012). Certainly, there exists a range of specializations. For example, Nepenthes ampullaria derives a significant proportion of its nitrogen from interception of falling leaf litter (Moran et al., 2003; Pavlović et al., 2011), while four other species (Nepenthes baramensis, Nepenthes lowii, Nepenthes rajah and Nepenthes macrophylla) sequester nutrients from mammalian excreta (Clarke et al., 2009; Chin et al., 2010; Grafe et al., 2011; Wells et al., 2011). Nepenthes albomarginata is a termite specialist (Moran et al., 2001; Merbach et al., 2002), and Nepenthes bicalcarata has a mutualistic nutritional association with the ant Camponotus schmitzi (Clarke and Kitching, 1995; Merbach et al., 2007; Bazile et al., 2012; Thornham et al., 2012). However, the majority of Nepenthes species studied thus far capture and digest a range of small arthropods, and many are particularly attractive to ants (e.g. Juniper et al.,

**Background and Aims** Nepenthes (Nepenthaceae, approx. 120 species) are carnivorous pitcher plants with a centre of diversity comprising the Philippines, Borneo, Sumatra and Sulawesi. Nepenthes pitchers use three main mechanisms for capturing prey: epicuticular waxes inside the pitcher; a wettable peristome (a collar-shaped structure around the opening); and viscoelastic fluid. Previous studies have provided evidence suggesting that the first mechanism may be more suited to seasonal climates, whereas the latter two might be more suited to perhumid environments. In this study, this idea was tested using climate envelope modelling.

**Methods** A total of 94 species, comprising 1978 populations, were grouped by prey capture mechanism (large peristome, small peristome, waxy, waxless, viscoelastic, non-viscoelastic, ‘wet’ syndrome and ‘dry’ syndrome). Nineteen bioclimatic variables were used to model habitat suitability at approx. 1 km resolution for each group, using Maxent, a presence-only species distribution modelling program.

**Key Results** Prey capture groups putatively associated with perhumid conditions (large peristome, waxless, viscoelastic and ‘wet’ syndrome) had more restricted areas of probable habitat suitability than those associated putatively with less humid conditions (small peristome, waxy, non-viscoelastic and ‘dry’ syndrome). Overall, the viscoelastic group showed the most restricted area of modelled suitable habitat.

**Conclusions** The current study is the first to demonstrate that the prey capture mechanism in a carnivorous plant is constrained by climate. Nepenthes species employing peristome-based and viscoelastic fluid-based capture are largely restricted to perhumid regions; in contrast, the wax-based mechanism allows successful capture in both perhumid and more seasonal areas. Possible reasons for the maintenance of peristome-based and viscoelastic fluid-based capture mechanisms in Nepenthes are discussed in relation to the costs and benefits associated with a given prey capture strategy.

**Key words:** Biogeography, carnivorous plants, climate envelope modelling, Nepenthes, Nepenthaceae, Palaeotropics, pitcher plant, capture mechanism.
Epicuticular waxes (function: trapping). In many Nepenthes species, the upper part of the inner pitcher wall is covered in one or more layers of epicuticular waxes (Riedel et al., 2003, 2007; Poppinga et al., 2010). These waxes can interfere with an insect’s foothold in a number of ways. First, wax crystals can detach from the top layer to clog the claws, thus denying traction. Secondly, the waxy surface is unwettable, denying a foothold to insects that rely on capillary forces for traction (Scholz et al., 2010). Finally, the microscopic roughness of the wax crystal layer itself may reduce the effective area available for traction by the prey’s adhesive pads (Scholz et al., 2010). In many Nepenthes species, epicuticular waxes work in conjunction with semi-lunate cells. These are modified stomatal guard cells that line the upper part of the inner pitcher wall. They present an anisotropic surface that denies traction to the claws of insects attempting to escape (Pant and Bhatnagar, 1976; Owen and Lennon, 1999; Gaume et al., 2002; Thornhill et al., 2008; Wang et al., 2009; Gorb and Gorb, 2011). In contrast to the action of the peristome, the waxy zone appears to operate equally well under both wet and dry conditions.

Viscoelastic pitcher fluid (function: retention). A number of Nepenthes species produce pitcher fluid with viscoelastic properties. Insects falling into this type of fluid find it impossible to extricate themselves, and quickly drown (Gaume and Di Giusto, 2009; Bonhomme et al., 2011).

There is considerable variation in the degree to which a given Nepenthes species employs each mechanism. Bauer et al. (2012a) divided the capture strategy of Nepenthes species into two broad groups: peristome based and wax based. In addition, Benz et al. (2012) demonstrated an inverse relationship between peristome width and extent of the waxy zone in eight Nepenthes species, suggesting disruptive selective pressure to adopt one of two capture mechanisms. Similarly, Bonhomme et al. (2011) established an inverse relationship between the viscoelasticity of pitcher fluid and the amount of wax produced in 23 Nepenthes species, once more suggesting disruptive selection. A potential driver of such selection is climate: Bauer et al. (2012a) raised the possibility that a perhumid climate might favour the peristome-based strategy, whereas drier or more seasonal climates might favour a wax-based strategy (see points 1 and 2, above). Bonhomme et al. (2011) suggested that the production of viscoelastic fluid (as opposed to waxes) favours the capture of volant prey, which are relatively more common than crawling prey such as ants, in montane habitats. Of greater relevance to the current study, they also demonstrated that the viscoelastic Nepenthes fluid retains its retentive properties, even when diluted by the heavy rainfall typical of such environments.

Therefore, we decided to test the idea that the employment of the various capture mechanisms described above might be driven by climatic variables. Specifically, we explored the possibility that peristome-based and viscoelastic capture strategies are restricted to perhumid environments, whereas the wax-based strategy is favoured in drier or more seasonal environments. We took a climate envelope modelling approach, using Maxent, a widely used species distribution modelling program (Phillips et al., 2004, 2006; Elith et al., 2011; http://www.cs.princeton.edu/~schapire/maxent/).

MATERIALS AND METHODS
Nepenthes species population localities and pitcher characteristics
Geographical co-ordinates were recorded for populations of 94 Nepenthes species (Table 1). This represents approx. 80 % of the species currently described (McPherson, 2009). A total of 1978 populations were recorded from Thailand, Laos, Vietnam, Cambodia, Peninsular Malaysia, Sumatra, Java, Borneo, the Philippine Archipelago and Sulawesi. The populations of 69 species were observed directly in the wild by one of us (C.C.); data on the remaining species were derived from examinations of herbarium material, cultivated plants and taxonomic descriptions (e.g. Danser, 1928). For many Nepenthes species with limited geographical distributions, the observations represent the entire currently known geographical range. Members of the Nepenthaceae are remarkable for high degrees of endemism; many are montane with restricted geographical ranges, often a single mountain or a small number of adjacent mountains (e.g. N. macrophylla, N. clipeata, N. aristolochioides; Clarke, 1997, 2001). Consequently, for 76 of our study species (approx. 80 %), only a small number of populations (<10) are currently known to exist. In contrast, a small number of species have very large geographical ranges, and a consequently larger number of identified populations. For example, 460 populations of N. gracilis were identified, spanning Thailand, Peninsular Malaysia, Sumatra, Borneo and Sulawesi; similarly, there were 342 populations of N. ampullaria identified, encompassing Thailand, Peninsular Malaysia, Sumatra and Borneo (Table 1). Initially, we considered randomly sub-sampling from the set of populations for these wide-ranging species, to limit any potential taxonomic bias in our results as a result of one or two species numerically dominating a given prey capture mechanism group.
TABLE 1. Summary of *Nepenthes* species used in the study, including pitcher characteristics

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of populations</th>
<th>Peristome size*</th>
<th>Wax</th>
<th>Viscoelastic fluid†</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nepenthes adnata</em></td>
<td>3</td>
<td>Small</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Nepenthes alata</em></td>
<td>17</td>
<td>Small</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Nepenthes alba</em></td>
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<td>Small</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Nepenthes albomarginata</em></td>
<td>27</td>
<td>Small</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Nepenthes ampullaria</em></td>
<td>342</td>
<td>Large</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Nepenthes andamanana</em></td>
<td>5</td>
<td>Small</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Nepenthes argenti</em></td>
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<td>Medium</td>
<td>Absent</td>
<td>Absent</td>
</tr>
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<td><em>Nepenthes aristolochioides</em></td>
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<td>Present</td>
</tr>
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<td>Medium</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Nepenthes bellii</em></td>
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<td>Medium</td>
<td>Absent</td>
<td>NA†</td>
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<td>Absent</td>
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<td>Absent</td>
</tr>
<tr>
<td><em>Nepenthes bongso</em></td>
<td>13</td>
<td>Large</td>
<td>Reduced</td>
<td>Present</td>
</tr>
<tr>
<td><em>Nepenthes boschiana</em></td>
<td>3</td>
<td>Large</td>
<td>Present</td>
<td>Absent</td>
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<tr>
<td><em>Nepenthes burdigaeae</em></td>
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<td>Large</td>
<td>Absent</td>
<td>Present</td>
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<tr>
<td><em>Nepenthes burkei</em></td>
<td>1</td>
<td>Large</td>
<td>Reduced</td>
<td>Absent</td>
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<tr>
<td><em>Nepenthes chang</em></td>
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<td>Present</td>
<td>Present</td>
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<td>Small</td>
<td>Present</td>
<td>NA</td>
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<td>Reduced</td>
<td>Present</td>
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<tr>
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<td>Reduced</td>
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<tr>
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<td>Present</td>
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<td>Present</td>
<td>Absent</td>
</tr>
<tr>
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<td>Absent</td>
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<td><em>Nepenthes eustachya</em></td>
<td>14</td>
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<td>Absent</td>
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<td>Medium</td>
<td>Reduced</td>
<td>Present</td>
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<tr>
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<td>Present</td>
<td>Absent</td>
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<td>Absent</td>
<td>Present</td>
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<tr>
<td><em>Nepenthes fusca</em></td>
<td>47</td>
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<tr>
<td><em>Nepenthes gloriosa</em></td>
<td>8</td>
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<td>Present</td>
<td>Present</td>
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<tr>
<td><em>Nepenthes glandulifera</em></td>
<td>1</td>
<td>Small</td>
<td>Reduced</td>
<td>Present</td>
</tr>
<tr>
<td><em>Nepenthes gracilis</em></td>
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<td>Present</td>
<td>Absent</td>
</tr>
<tr>
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<td>Absent</td>
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<tr>
<td><em>Nepenthes gymnamphora</em></td>
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<td>Present</td>
<td>Absent</td>
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<td>Absent</td>
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<tr>
<td><em>Nepenthes hirsuta</em></td>
<td>19</td>
<td>Small</td>
<td>Reduced</td>
<td>Absent</td>
</tr>
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<td>Small</td>
<td>Present</td>
<td>Absent</td>
</tr>
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<td>Present</td>
<td>Absent</td>
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<tr>
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<td>Small</td>
<td>Reduced</td>
<td>Present</td>
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<td><em>Nepenthes izamiae</em></td>
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<td>Present</td>
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<tr>
<td><em>Nepenthes jacquelineae</em></td>
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<td>Absent</td>
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<tr>
<td><em>Nepenthes jamban</em></td>
<td>1</td>
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<td>Absent</td>
<td>Present</td>
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<tr>
<td><em>Nepenthes kampotiana</em></td>
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<td>Small</td>
<td>Present</td>
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<td><em>Nepenthes kerrii</em></td>
<td>2</td>
<td>Small</td>
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<td>Absent</td>
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<td><em>Nepenthes kongkandana</em></td>
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<td>Small</td>
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<td>Absent</td>
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<tr>
<td><em>Nepenthes lingulata</em></td>
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<td>Absent</td>
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<td><em>Nepenthes longifolia</em></td>
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<td>Present</td>
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<td><em>Nepenthes macfarlanei</em></td>
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<td>Present</td>
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<td>Medium</td>
<td>Present</td>
<td>Present</td>
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<tr>
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<td>Small</td>
<td>Reduced</td>
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<tr>
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<td>Present</td>
<td>NA</td>
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<tr>
<td><em>Nepenthes maxima</em></td>
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<td>Medium</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td><em>Nepenthes merrilliana</em></td>
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<td>Absent</td>
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<td><em>Nepenthes mirabilis</em></td>
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<td><em>Nepenthes malaeensis</em></td>
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<td><em>Nepenthes maradensis</em></td>
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<tr>
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<td>Reduced</td>
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</tr>
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<td>Medium</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td><em>Nepenthes philippinensis</em></td>
<td>4</td>
<td>Small</td>
<td>Present</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Continued
(see below for details regarding groups). However, to have downsampled would have created false absences, which would have reduced the accuracy of the models used. It is important to stress that for the purposes of this study, we were focused not on individual species, but on the capture mechanism. Therefore, if a population of plants using a given mechanism was known and located, it was included in the analysis. For our purposes, the specific status of a plant was less important than the fact that, for instance, a population of *Nepenthes* conforming to a given capture mechanism group or syndrome (see below) was present at a given location. For each species, the following pitcher attributes were quantified.

1. **Peristome size.** We adopted the approach of Bauer *et al.* (2012a) in assigning peristome width classes based on the relationship of peristome width to pitcher length. The relative peristome size of 65 species was derived from fig. 8 of Bauer *et al.* (2012a); values for the remaining 29 species in the current study were derived from field measurements by C.C. Species were then assigned to one of three peristome size categories (small, medium and large). These correspond to the following categories of peristome width in fig. 8 of Bauer *et al.* (2012a): small = –20 to –10; medium = –5 to +5; large = +10 to +25. These numerical values refer to the magnitude of the residuals from the peristome width/pitcher length regression relationship: positive values denote larger than average peristomes; negative values, smaller than average (Table 1). We did not apply a correction for phylogeny (see Cheverud *et al.*, 1985) to the peristome size measurements, since Bauer *et al.* (2012a) found no significant difference between phylogenetically corrected and uncorrected morphological data for the 65 species that they examined in their study (i.e. no detectable phylogenetic signal with regards to morphological traits). Since their 65 species account for 70% of the total number of species used in the current study, we believe it highly unlikely that the other species we used would depart from this pattern in any significant way.

2. **Presence/absence of wax.** We used species data describing wax production from Bonhomme *et al.* (2011), Bauer *et al.* (2012a) and Benz *et al.* (2012), as well as direct observation of specimens in the field by C.C. (Table 1). For the reasons outlined above, we did not apply a phylogenetic correction to the wax data-set.

3. **Presence/absence of viscoelastic pitcher fluid.** For each species, the presence or absence of viscoelastic pitcher fluid was noted. Data for 23 species were taken from the data set presented by Bonhomme *et al.* (2011); the remaining data were from direct field observations by C.C. A potential source of error in our approach is that assessment of
viscoelasticity was made based on direct observation of pitcher fluid, rather than rheological measurement.

**Nepenthes** undergo ontogenic change in pitcher morphology. Young plants typically produce terrestrial pitchers that are ovoid in shape; as the plants grow, aerial pitchers are produced. These are usually more elongate in form. In a number of species, this ontogenic shift in gross morphology is accompanied by changes in wax production, fluid viscoelasticity and/or relative width of the peristome. In such cases, we took the approach that if, for example, viscoelastic fluid was produced by either pitcher form of a given species, then that species was classified as employing viscoelastic fluid (Table 1). The same approach was taken regarding ontogenic changes in wax production and peristome size.

**Nepenthes capture mechanism groupings**

Based on the three attributes outlined above, we grouped the species into eight putative capture mechanism groups (hereafter called groups), summarized in Table 2. Six of the groups focus on a single pitcher characteristic (peristome size, viscoelasticity of fluid and waxiness). The final two groups represent combinations of these characteristics: the ‘wet’ syndrome comprises species that possess large peristomes, and produce no wax; the ‘dry’ syndrome comprises species with small peristomes, wax and non-viscoelastic fluid. It might have been considered optimal to have incorporated only species that produce viscoelastic fluid into the ‘wet’ syndrome, based on the observations of Bonhomme et al. (2011). However, only seven species possessed pitchers with large peristomes, viscoelastic fluid and no wax, and the total number of populations for this combination of traits was only 18, far below the threshold of 80 required for meaningful modelling using Maxent (Elith et al., 2011). The large and medium peristome size classes were combined in the large peristome group and ‘wet’ syndrome. This is because there appears to be little difference between the two peristome size categories from a functional viewpoint. For example, both N. bicalcarata (large peristome category) and N. rafflesiana (medium peristome category) employ peristome aquaplaning as a prey-trapping mechanism (Bohn and Federle, 2004; Bauer et al., 2008).

**Climate variables**

We considered 19 bioclimatic variables as potential predictors of *Nepenthes* group distributions (summarized in Table 3). These variables are freely available at 30 arc-seconds (approx. 1 km) resolution from the WorldClim data set version 1.4, Release 3 (Hijmans et al., 2005; http://www.worldclim.org/bioclim). This data set comprises gridded precipitation and temperature data based on monthly measurements from globally distributed weather stations. The data are averaged from the years 1950–2000 to generate a single 50 year ‘climate surface’ for each month. The resulting surfaces are then utilized to generate bioclimatic variables (e.g. mean temperature of the wettest quarter), which are considered more meaningful biologically than monthly measurements for defining the environmental tolerances of a species (Graham and Hijmans, 2006; Murienne et al., 2009). These variables have been widely used to predict plant species distributions in other habitat modelling studies (e.g. Kumar et al., 2006; Guisan et al., 2007a, b; Murienne et al., 2009; Kumar and Stohlgren, 2009). We did not use monthly meteorological data due to differences in seasonal weather patterns over the extensive geographical area covered in the study. For instance, in western Java, the period of highest rainfall occurs between December and February; in contrast, the highest rainfall in northern Thailand occurs during September (source: Meteorological Service of Singapore; http://www.weather.gov.sg). To have used monthly data might have prevented the model from associating particular climate variables with a highly correlated capture group, thus reducing model accuracy.

**Climate envelope model development and evaluation**

Using the bioclimatic variables discussed above, we employed Maxent V. 3.3.3k (Phillips et al., 2004) to model group distributions in our geographical area of interest. This encompassed Thailand, Laos, Vietnam, Cambodia, Peninsular Malaysia, Sumatra, Java, Borneo, the Philippines and Sulawesi. Maxent is a maximum entropy-based machine learning program that estimates the probability distribution for the occurrence of a species based on constraints of environmental variables on species distribution (Phillips, 2006; Phillips et al., 2006; Elith et al., 2011). The current study modelled distributions of capture mechanisms (Table 2) rather than individual species, and generated an estimate of the probability of the presence of a given group that varied from 0 to 1, where 0 was the lowest and 1 the highest probability. The advantage of this modelling approach is that it only requires presence data and environmental variable layers (continuous or categorical) for the study area (Phillips and Dudík, 2008). Moreover, Maxent has been found to produce the most accurate models among many different species distribution modelling methods (Elith et al., 2006; Hijmans and Graham, 2006; Townsend Peterson et al., 2007). Given that we only considered continuous environmental variables, and the lowest number of samples within a single group

<table>
<thead>
<tr>
<th>Group</th>
<th>Peristome</th>
<th>Wax</th>
<th>Viscoelastic fluid</th>
<th>No. of species</th>
<th>No. of populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small peristome</td>
<td>Small</td>
<td>Any</td>
<td>Any</td>
<td>41</td>
<td>1098</td>
</tr>
<tr>
<td>Large peristome</td>
<td>Large or medium</td>
<td>Any</td>
<td>Any</td>
<td>53</td>
<td>881</td>
</tr>
<tr>
<td>Waxy</td>
<td>Any</td>
<td>Yes</td>
<td>Any</td>
<td>56</td>
<td>1170</td>
</tr>
<tr>
<td>Waxless</td>
<td>Any</td>
<td>No</td>
<td>Any</td>
<td>20</td>
<td>422</td>
</tr>
<tr>
<td>Viscoelastic</td>
<td>Any</td>
<td>Any</td>
<td>Yes</td>
<td>33</td>
<td>460</td>
</tr>
<tr>
<td>Non-viscoelastic</td>
<td>Any</td>
<td>Any</td>
<td>No</td>
<td>56</td>
<td>1505</td>
</tr>
<tr>
<td>‘Dry’ syndrome</td>
<td>Small</td>
<td>Yes</td>
<td>No</td>
<td>29</td>
<td>1010</td>
</tr>
<tr>
<td>‘Wet’ syndrome</td>
<td>Large or medium</td>
<td>No</td>
<td>Any</td>
<td>18</td>
<td>416</td>
</tr>
</tbody>
</table>

**Table 2. Putative Nepenthes prey capture mechanism groups modelled in the study**
### Table 3. Results of Maxent models for putative prey capture mechanism groups in Nepenthes

<table>
<thead>
<tr>
<th>Bioclimatic variable*</th>
<th>Large peristome</th>
<th>Small peristome</th>
<th>Waxy</th>
<th>Waxless</th>
<th>Viscoelastic</th>
<th>Non-viscoelastic</th>
<th>‘Wet’ syndrome</th>
<th>‘Dry’ syndrome</th>
<th>Test AUC**</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIO1 (annual mean temperature)</td>
<td>0.00</td>
<td>4.90</td>
<td>0.00</td>
<td>0.00</td>
<td>0.85</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.81</td>
</tr>
<tr>
<td>BIO2 (mean diurnal temperature range)</td>
<td>10.66</td>
<td>13.03</td>
<td>9.92</td>
<td>8.11</td>
<td>4.85</td>
<td>8.91</td>
<td>2.51</td>
<td>0.00</td>
<td>10.77</td>
</tr>
<tr>
<td>BIO3 (isothermality)†</td>
<td>8.51</td>
<td>10.37</td>
<td>7.95</td>
<td>4.96</td>
<td>0.00</td>
<td>7.05</td>
<td>0.00</td>
<td>0.00</td>
<td>7.66</td>
</tr>
<tr>
<td>BIO4 (temperature seasonality)‡</td>
<td>0.40</td>
<td>0.23</td>
<td>0.20</td>
<td>0.21</td>
<td>0.81</td>
<td>0.27</td>
<td>0.39</td>
<td>0.40</td>
<td>0.00</td>
</tr>
<tr>
<td>BIO5 (maximum temperature of warmest month)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>BIO6 (minimum temperature of coldest month)</td>
<td>9.10</td>
<td>0.69</td>
<td>1.40</td>
<td>9.46</td>
<td>6.50</td>
<td>5.11</td>
<td>14.37</td>
<td>0.31</td>
<td>0.00</td>
</tr>
<tr>
<td>BIO7 (annual temperature range)§</td>
<td>16.49</td>
<td>11.72</td>
<td>7.88</td>
<td>12.72</td>
<td>6.45</td>
<td>10.97</td>
<td>0.77</td>
<td>0.00</td>
<td>8.24</td>
</tr>
<tr>
<td>BIO8 (mean temperature of wettest quarter)</td>
<td>16.51</td>
<td>17.12</td>
<td>22.50</td>
<td>9.59</td>
<td>27.73</td>
<td>18.58</td>
<td>10.98</td>
<td>16.27</td>
<td>0.00</td>
</tr>
<tr>
<td>BIO9 (mean temperature of driest quarter)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>BIO10 (mean temperature of warmest quarter)</td>
<td>1.31</td>
<td>7.55</td>
<td>10.11</td>
<td>11.10</td>
<td>0.17</td>
<td>13.86</td>
<td>12.95</td>
<td>12.22</td>
<td>0.00</td>
</tr>
<tr>
<td>BIO11 (mean temperature of coldest quarter)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>BIO12 (annual precipitation)</td>
<td>9.56</td>
<td>9.37</td>
<td>12.14</td>
<td>5.53</td>
<td>18.32</td>
<td>7.13</td>
<td>9.42</td>
<td>8.52</td>
<td>0.00</td>
</tr>
<tr>
<td>BIO13 (precipitation of wettest month)</td>
<td>1.12</td>
<td>1.09</td>
<td>0.76</td>
<td>1.60</td>
<td>0.88</td>
<td>1.08</td>
<td>2.81</td>
<td>0.62</td>
<td>0.00</td>
</tr>
<tr>
<td>BIO14 (precipitation of driest month)</td>
<td>8.18</td>
<td>2.54</td>
<td>3.49</td>
<td>13.29</td>
<td>4.36</td>
<td>7.10</td>
<td>18.39</td>
<td>5.00</td>
<td>0.00</td>
</tr>
<tr>
<td>BIO15 (precipitation seasonality)¶</td>
<td>0.60</td>
<td>1.70</td>
<td>0.94</td>
<td>0.09</td>
<td>5.62</td>
<td>0.05</td>
<td>0.72</td>
<td>0.10</td>
<td>0.00</td>
</tr>
<tr>
<td>BIO16 (precipitation of wettest quarter)</td>
<td>16.43</td>
<td>14.24</td>
<td>16.99</td>
<td>17.04</td>
<td>21.17</td>
<td>15.37</td>
<td>21.60</td>
<td>12.33</td>
<td>0.00</td>
</tr>
<tr>
<td>BIO17 (precipitation of driest quarter)</td>
<td>0.07</td>
<td>3.79</td>
<td>2.51</td>
<td>3.52</td>
<td>0.62</td>
<td>2.21</td>
<td>2.42</td>
<td>3.18</td>
<td>0.00</td>
</tr>
<tr>
<td>BIO18 (precipitation of warmest quarter)</td>
<td>1.06</td>
<td>1.55</td>
<td>3.10</td>
<td>2.75</td>
<td>1.27</td>
<td>2.19</td>
<td>1.77</td>
<td>3.32</td>
<td>0.00</td>
</tr>
<tr>
<td>BIO19 (precipitation of coldest quarter)</td>
<td>0.00</td>
<td>0.11</td>
<td>0.10</td>
<td>0.03</td>
<td>0.38</td>
<td>0.12</td>
<td>0.31</td>
<td>0.31</td>
<td>0.00</td>
</tr>
<tr>
<td>Test AUC**</td>
<td>0.81</td>
<td>0.77</td>
<td>0.77</td>
<td>0.82</td>
<td>0.86</td>
<td>0.77</td>
<td>0.83</td>
<td>0.80</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* Bioclimatic variables are in bold if permutation importance is ≥5% for any group.

† Isothermality is a measure of diurnal temperature range compared with annual temperature range, calculated as: (BIO2/BIO7) × 100.

‡ (Standard deviation) × 100.

§ (BIO5–BIO6).

¶ Coefficient of variation of precipitation.

** AUC: area under curve, a measure of model performance. A value of 0.5 represents a model that performs no better than random; a value of 1 represents a perfect fit.
(n = 378) was almost 5-fold greater than the acceptable minimum of 80 observations (Elith et al., 2011), we were able to use the product feature type for our model execution. This feature uses the product of pairs of continuous environmental variables, together with the linear feature of each variable, to constrain the co-variance of the respective variables to the empirical values, thereby incorporating interactions between predictor variables into the suitable habitat projections (Phillips et al., 2006). Additionally, the default Maxent model parameters (regularization multiplier = 1 and convergence threshold = $10^{-5}$) were used. However, to decrease the chance of the model over- or underpredicting suitable habitat for each group, we increased the maximum number of iterations from 500 (the default value) to 5000, to allow the model a sufficient number of iterations for convergence.

When defining the environmental tolerances of the groups examined in this study, it was important to consider potential geographic sampling bias within the occurrence data, given differing degrees of accessibility between sampling locations. For example, a potential source of bias in species distribution modelling based on presence-only data is the tendency to over-represent observations close to roads and other means of access to a given area. Phillips et al. (2009) suggested a method to correct for sampling bias, by selecting background data with similar sampling bias to the occurrence data. Following the steps outlined in Phillips (2006) and Young et al. (2011), a bias file was generated using the smallest administrative unit (recorded as district, county or municipality) for all countries in our study area. The geographic space of an administrative unit was included in the bias file if an observation fell within the unit. By including the bias file, Maxent sampled the default 10,000 pseudo-absences from the collection of small-scale geographic units where observations were recorded, rather than from the entire prediction area.

Finally, for model evaluation, the random test percentage was set at 30, so that 70 % of the observations for a given group were randomly selected as a training data set and the remaining 30 % were used for model testing. Model fit was evaluated by the widely used area under the curve (AUC) of the receiver operating characteristic (ROC), which represents the probability that the model classifier will correctly identify a randomly chosen true presence (Fielding and Bell, 1997; Fawcett, 2006). The AUC is a threshold-independent measure of model performance that ranges from 0 to 1; a value of 0.5 represents a model that performs no better than random, whilst 1 is maximally predictive. To evaluate model performance further, the replicates option was set at 10 so that the selection of testing data and calculation of AUC values were repeated. Therefore, final model performance was evaluated with the mean AUC for each group over the ten replicates. Similarly suitable habitat (in terms of the climate envelope) for each group was displayed as the average projected probability of presence across the ten replicates. Although the Maxent parameter settings listed above were constant for all groups, each was modelled and evaluated individually. Graphical outputs of the model for each group were produced using the ArcMap V.10 software package (Esri Inc., Redlands, CA, USA).

**Evaluation of bioclimatic variables as predictors of occurrence**

Maxent provides several methods for quantifying the relative contribution of each variable to the model. We used ‘permutation importance’ to investigate which bioclimatic variables were most important for predicting geographical distribution of a given group. This is a measure of decrease in training AUC (expressed as a percentage) resulting from randomly permuting values of a given variable during the training phase of model development. The decrease in training AUC is in proportion to the importance of that variable to the model (Phillips, 2006). In addition, we compared the relative magnitude of important variables between group pairs (large peristome vs. small peristome, waxy vs. waxless, viscoelastic vs. non-viscoelastic, and ‘wet’ syndrome vs. ‘dry’ syndrome), to allow identification of variables that might be important in delimiting suitable habitat for a given group. For each bioclimatic variable, permutation importance values were summed for each pair of groups. The relative contribution from each member of the pair to the total was then calculated as a percentage, as follows:

\[
\% \text{ importance of bioclimatic variable to group } A = \left[ \frac{\text{PIA}}{\text{PIA} + \text{PIB}} \right] \times 100
\]

Where PIA and PIB denote permutation importance of a given bioclimatic variable to groups A and B, respectively. We considered variables with permutation importance values ≥ 5 % as important; the comparisons included only those variables with values at or above this threshold for at least one group in each pair.

**RESULTS**

**Model evaluation**

Model evaluation statistics for group predictions are presented in Table 3. Mean AUC values were consistently high, ranging from 0.77 to 0.86. A high AUC value indicates a high level of model accuracy: a value of 0.75 means that 75 % of the time a random sample from presence predictions will have a score greater than a random selection from absence predictions across all available probability thresholds. Therefore, an AUC value of 0.5 indicates a random predictor, and values between 0.5 and 0.6 are generally considered indicative of a failed model (Fawcett, 2006). Overall, groups with capture mechanisms predicted to be associated with wet environments (large peristome, waxless, viscoelastic and ‘wet’ syndrome) have slightly higher AUC values than groups predicted to occur in drier or more seasonal environments (small peristome, waxy, non-viscoelastic and ‘dry’ syndrome; Table 3).

For each group, graphical representations of modelled habitat suitability are presented in Fig. 1A–H. Probability of habitat suitability is plotted on a colour scale, where grey represents values of 0–0.1 and dark blue represents values of 0.9–1. In general, the groups putatively associated with perhumid conditions (large peristome, waxless, viscoelastic and ‘wet’ syndrome; Fig. 1B, D, F, H) show less extensive areas of probable habitat suitability than do those associated putatively with less humid conditions (small peristome, waxy, non-viscoelastic and ‘dry’ syndrome; Fig. 1A, C, E, G). The most restricted area of suitable habitat is associated with the viscoelastic group (Fig. 1F).
FIG. 1. Probability of habitat suitability for eight putative *Nepenthes* prey capture mechanism groups, modelled from 19 bioclimatic variables using Maxent. Probability is mapped by colour, from grey (0–0.1) to dark blue (0.9–1). Scale bar = 1000 km.
Bioclimatic variable importance

Of the 19 bioclimatic variables used in the models, two produced permutation importance values of zero for all models, i.e. they contributed no useful information to account for the observed distributions: mean temperature of the driest quarter (BIO9) and mean temperature of the coldest quarter (BIO11; Table 3). An additional six bioclimatic variables produced values of <5 % for all groups, essentially contributing no useful information to the models: temperature seasonality (BIO4), maximum temperature of the warmest month (BIO5), precipitation of the wettest month (BIO13), precipitation of the driest quarter (BIO17), precipitation of the warmest quarter (BIO18) and precipitation of the coldest quarter (BIO19; Table 3). Conversely, three variables were important in informing the model across all groups: mean temperature of the wettest quarter (BIO8), annual precipitation (BIO12) and precipitation of the wettest quarter (BIO16), which produced high permutation importance values (>5 %), ranging from 5.5 % to as high as 27.7 %. The remaining eight bioclimatic variables produced values that varied across groups and, therefore, provide insight into the differential influence of climatic factors between them (see below).

Comparisons of bioclimatic variable importance for habitat suitability between groups

There are discernible patterns of differences in permutation importance for habitat suitability between the groups putatively associated with perhumid conditions and those putatively associated with drier environments (Fig. 2). Compared with the small peristome group, the large peristome group is influenced to a higher degree by minimum temperature of the coldest month (BIO6), precipitation of the driest month (BIO14) and annual temperature range (BIO7). Conversely, the small peristome group is more strongly influenced by the mean temperature of the warmest quarter (BIO10; Fig. 2A). The presence of wax is highly influenced by mean temperature of the wettest quarter (BIO8), annual precipitation (BIO12) and isothermality (BIO3) compared with the absence of wax. Conversely, the waxless group is influenced more by annual temperature range (BIO7), precipitation of the driest month (BIO14) and minimum temperature of the coldest month (BIO6; Fig. 2B). Habitat suitability for the viscoelastic group is highly influenced by seasonality of precipitation (BIO15), annual precipitation (BIO12), mean temperature of the wettest quarter (BIO18), precipitation of the wettest quarter (BIO16) and minimum temperature of the coldest month (BIO6) compared with the non-viscoelastic group. Conversely, the latter is more highly influenced by isothermality (BIO3), mean temperature of the wettest quarter (BIO10), mean diurnal temperature range (BIO2), annual temperature range (BIO7) and precipitation of the driest month (BIO14; Fig. 2C). Comparing the ‘wet’ and ‘dry’ syndromes, the former is influenced more by the minimum temperature of the coldest month (BIO6), precipitation of the driest month (BIO14) and precipitation of the wettest quarter (BIO16). Conversely, annual mean temperature (BIO1), isothermality (BIO3), annual temperature range (BIO2), mean diurnal temperature range (BIO2) and mean temperature of the wettest quarter (BIO8) are more important for the ‘dry’ syndrome (Fig. 2D).

DISCUSSION

With geographical range extending from Madagascar to New Caledonia, Nepenthaceae is a successful and diverse carnivorous family. Nepenthes can be found from sea level to >3000 m
elevation, on a variety of substrates (Clarke, 1997). *Nepenthes* occur in both perhumid and seasonal tropical environments, and the range of pitcher morphologies is striking, even to the casual observer. This diversity of pitcher morphology is mirrored by the range of nitrogen sources exploited, including bat excreta, leaf litter and arthropods (e.g. Moran et al., 2003; Di Giusto et al., 2008; Grafe et al., 2011; Pavlović et al., 2011). Yet, despite this wide range of pitcher structure and function, we detected a consistent, large-scale pattern defining the relationship between bioclimatic variables and prey capture mechanism.

Peristome

Bohn and Federle (2004) and Bauer et al. (2008) demonstrated the profound reliance of peristome function on ambient moisture in *Nepenthes* species that rely on this structure as the primary trapping mechanism. The current study confirms that *Nepenthes* species employing peristome-based prey capture are generally restricted to perhumid regions. As Fig. 1 demonstrates, habitat suitable for the large peristome group is considerably less extensive than that for the small peristome group. It is confined largely to perhumid regions, notably the eastern Philippines, western Borneo, western Sumatra and eastern Peninsular Malaysia. In contrast, habitat suitable for the small peristome group is much less geographically constrained, and extends into regions with more seasonal climates, both north and south of the Equator.

The relative importance of individual bioclimatic factors to the Maxent models also provides clues to climatic constraints on peristome use. Compared with the small peristome group, habitat suitable for the large peristome group is influenced more by minimum temperature of the coldest month, annual temperature range and precipitation of the driest month. The first two variables could be considered pertinent with regards to montane climates (a large proportion of species in the large peristome group are montane/sub-montane in habit); the last variable suggests a constraint with regards to periods of low moisture availability, further supporting the idea that peristome-based capture is centred on perhumid regions.

Wax

It is evident from Fig. 2A and B that there is a reciprocal pattern of relative importance of bioclimatic variables between peristome-based capture and wax-based capture. The three bioclimatic variables identified above as important in the distribution of the large peristome group (minimum temperature of the coldest month, annual temperature range and precipitation of the driest month) are also the three most influential in the distribution of the waxless group. Similarly, the geographical range of suitable habitat is more restricted for the waxless group than for the waxy group. The maps for the small peristome and waxy groups are almost identical, as are those for the large peristome and waxless groups.

Given the previously noted inverse relationship between wax production and peristome size (Bauer et al., 2012a; Benz et al., 2012), this finding is perhaps not surprising. The graphical outputs from the models also confirm the prediction that species using wax as a capture mechanism would be less confined to perhumid conditions than those relying on a peristome-based strategy. From a functional viewpoint, this also makes intrinsic sense, since the wax-based capture mechanism does not require critical levels of ambient moisture, in contrast to peristome-based capture.

Viscoelastic fluid

Of the eight habitat models derived here, that for the viscoelastic group appears to be the most tightly constrained by climate, as evidenced by the highly limited geographical extent of suitable habitat. Bonhomme et al. (2011) noted the tendency for species with viscoelastic pitcher fluid to be montane in habit, and this is corroborated by the distribution of suitable habitat in the current study. They also suggested that the abundance of volant prey relative to crawling prey in tropical montane environments favours the deployment of viscoelastic fluid rather than waxes. Their assertion was based on experimental testing of the effects of wax and viscoelastic fluid on insect traction. They demonstrated that wax is a more effective mechanism for preventing escape of ants, whereas viscoelastic fluid is effective for both ants and flies. Further, the authors determined that an inverse relationship (similar to that between peristome and wax) exists between deployment of viscoelastic fluid and deployment of wax. This relationship would account for the almost identical areas of suitable habitat modelled for the waxy and non-viscoelastic groups. It has been shown that even when highly diluted with water (as might be the case with rainwater in montane and other perhumid environments), *Nepenthes* viscoelastic fluid retains its prey-retentive properties (Gaume and Forterre, 2007; Bonhomme et al., 2011). Thus, we might expect the production of viscoelastic fluid to be associated with wetter habitats, and the findings of the current study appear to support this idea. The results of the paired group comparison show that three of the five important bioclimatic variables for the viscoelastic group pertain to precipitation. In contrast, only one of the five important variables for the non-viscoelastic group is based on precipitation; the rest are all temperature related.

‘Wet’ and ‘dry’ syndromes

Given that the putative ‘wet’ and ‘dry’ syndromes are based on combinations of the individual prey capture mechanisms dealt with above, it is perhaps not surprising that the modelled habitat suitability patterns closely match them, with the former being restricted to perhumid regions and the latter extending into more seasonal regions. Of the three bioclimatic variables shown to be important to the ‘wet’ syndrome model in the paired comparison, two are precipitation related; in contrast, all five of the variables shown to be important to the ‘dry’ syndrome model are temperature related.

No evidence for edaphic influences on group distributions

Climate is not the only determinant of the geographical distributions of organisms. For plants, edaphic factors can play a critical role in determining where a given species is likely to occur. As with other carnivorous plant taxa, *Nepenthes* are restricted to nutrient-poor substrates (Givnish et al., 1984). There is a variety of such substrates available across the geographical range of *Nepenthes*, and many species are restricted to one or more of them. For example, *N. rafflesiana* is typically found growing on silica-derived podzols, *N. bicalcarata* occurs predominantly
on peat-derived soils, *Nepenthes fazialiana* is restricted to limestone outcrops, *N. rajah* grows on serpentine soils derived from ultramafic bedrock, and *Nepenthes sumatrana* occurs on sandstone outcrops (Clarke, 1997, 2001). However, although soil type may play an important role in the distribution of individual *Nepenthes* species, it does not appear to be a factor in the distribution of capture mechanisms examined in the current study. Evidence for this is found in the five species mentioned above: although each is restricted to a particular soil type, all are members of the large peristome group. Similar examples can be found for the other groups. Therefore, we suggest that although the distribution of *Nepenthes* as a genus (as well as individual species, as highlighted above) may be constrained by climate and soil type, the geographical distribution of the three major capture mechanisms can be explained largely by climatic variables alone. *Nepenthes* species employing peristome-based or viscoelastic mechanisms are more restricted to perhumid areas (including montane environments); in contrast, those employing wax-based capture are less constrained by moisture regime, and can successfully inhabit more seasonal environments.

Reasons for maintenance of multiple prey capture mechanisms in Nepenthaceae

The current study has established that the wax-based capture strategy in *Nepenthes* facilitates carnivory in both perhumid and more seasonal environments. If the wax-based strategy is effective over such a large geographical range, why are the other mechanisms (large peristome and viscoelastic fluid) maintained in *Nepenthes* at all? There is a consensus that the ‘ancestral’ state of *Nepenthes* pitchers is represented by a wax-based capture strategy, and that this strategy has been lost independently in several clades (Meimberg and Heubl, 2006; Gaume and Di Giusto, 2009; Bauer et al., 2012a). What might be the evolutionary drivers of wax loss and the consequent adoption of alternative strategies? One possibility is differential effectiveness of the trapping mechanisms on different prey taxa: Bonhomme et al. (2011) demonstrated that viscoelastic fluid was more effective than wax in retaining volant prey. They also suggested that volant prey are more prevalent than crawling prey in montane habitats, thus favouring deployment of viscoelastic fluid. However, a discussion of possible prey specialization with regards to bioclimatic variables is beyond the scope of the current study.

Another possibility is that there exists a cost differential between wax-based and alternative strategies. Regardless of trapping mechanism, the production of pitchers incurs a cost to the plant. There is a quantifiable ‘opportunity cost’ associated with producing a foliar structure that delivers sub-optimal photosynthetic performance. For example, pitchers of *Nepenthes talangensis* exhibit significantly reduced rates of photosynthesis (*A*$_{N}$) and quantum yield of photosystem II (*Φ*$_{PSII}$) compared with laminae (Pavlović et al., 2009). Additionally, some components of the pitcher are more expensive than others to produce. The construction costs of a plant organ such as a leaf can be defined as the amount of photosynthate required to produce a unit weight of biomass (Poorter and De Jong, 1999; Villar et al., 2006). Simple compounds such as cellulose are relatively inexpensive to produce; however, synthesis of secondary compounds (e.g. lignin and tannins) incurs higher construction costs (Poorter and De Jong, 1999; Villar et al., 2006; Osunkoya et al., 2007, 2008). The peristome is a rigid, structurally reinforced region of the pitcher, and this rigidity is due to the incorporation of these more expensive secondary compounds. The viscoelasticity of pitcher fluid results from the presence of long-chain polymers (Bonhomme et al., 2011) the relative construction costs of which have yet to be determined. The waxes produced by *Nepenthes* pitchers are rich in primary alcohols and very long chain aldehydes (Riedel et al., 2003, 2007). Waxes incur significantly higher construction costs than secondary compounds such as tannins and lignin (Poorter and De Jong, 1999; Martínez et al., 2002). The inner walls of *N. albomarginata* pitchers produce >35 μg cm$^{-2}$ of wax (Riedel et al., 2007), which would, other factors being equal, incur considerably higher construction costs than pitchers of waxless congeners. Therefore, we suggest that the reason that wax has been lost independently from several clades over time is probably due to the costs of production outweighing the benefits gained by its deployment in a particular environment. If the less resource-costly peristome is as effective as wax in perhumid regions, it would be to a plant’s advantage to reduce or cease expensive wax production to maximize net benefit. This idea is in agreement with the findings of Bonhomme et al. (2011), Benz et al. (2012) and Bauer et al. (2012a): in *Nepenthes*, there is a strong reciprocal relationship between the use of wax and the deployment of alternative capture mechanisms. It is also in agreement with the findings of the current study, with regards to the geographical distributions of wax-based vs. alternative prey capture mechanisms. To conclude, we suggest that climate is a strong candidate as the agent of the observed disruptive selection between wax-based and either peristome-based or viscoelastic fluid-based capture syndromes in *Nepenthes*.

ACKNOWLEDGEMENTS

We thank Royal Roads University for a grant to support the study, B. Hawkins for material help, and A. Moran for reviewing the manuscript. Author contributions: J.M., C.C. and L.C. conceived the study; C.C. collected the data; L.G. and J.M. analysed the data; J.M. led the writing with contributions from L.G., C.C. and L.C. L.G. constructed the figures. Two anonymous reviewers helped to improve the manuscript considerably.

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