



REVIEW ARTICLE

The evolution of alternative mechanisms that promote outcrossing in Annonaceae, a self-compatible family of early-divergent angiosperms

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Annonaceae flowers are generally hermaphroditic and show high levels of outcrossing, but unlike many other early-divergent angiosperms lack a self-incompatibility mechanism. We reassess the diversity of mechanisms that have evolved to avoid self-pollination in the family. Protogyny occurs in all hermaphroditic flowers in the family, preventing autogamy but not geitonogamy. Herkogamy is rare in Annonaceae and is likely to be less effective as beetles move randomly around the flowers in search of food and/or mates. Geitonogamy is largely avoided in Annonaceae by combining protogyny with floral synchrony, manifested as either pistillate/staminate-phase synchrony (in which pistillate-phase and staminate-phase flowers do not co-occur on an individual) or heterodichogamy (in which two phenologically distinct and reproductively isolated morphs coexist in populations). Unisexual flowers have evolved independently in several lineages, mostly as andromonoecy (possibly androdioecy). Functionally monoecious populations have evolved from andromonoecious ancestors through the loss of staminate function in structurally hermaphroditic flowers. This has been achieved in different ways, including incomplete pollen/stamen development and delayed anther dehiscence. Angiosperms display an enormous diversity of mechanisms to promote xenogamy, many of which are easily overlooked without fieldwork. Floral phenology is particularly important, especially cryptic differences in timing of organ maturation or abscission. © 2013 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2014, **174**, 93–109.

ADDITIONAL KEYWORDS: autogamy – delayed anther dehiscence – dichogamy – dioecy – floral synchrony – geitonogamy – herkogamy – incomplete pollen development – protogyny – self-incompatibility – xenogamy.

INTRODUCTION

Breeding systems in sexually reproducing populations are governed by the proportion of inbreeding and outcrossing among individuals. In flowering plants this balance is influenced by various factors, including population size, the distribution of sex organs within the individual and flower, floral morphology and phenology, pollination mechanisms, and pollinator behaviour (Clegg, 1980; Loveless & Hamrick, 1984; Dafni, 1992; Richards, 1997). The manner in which this balance is achieved in early-divergent angiosperms is of particular interest given their phylogenetic position and hence their impact on infer-

ences on broader-scale evolutionary transitions in angiosperms.

Biochemically mediated self-incompatibility (SI), in which sexually reproducing hermaphroditic individuals are not capable of self-fertilization, is widespread among angiosperms, occurring in more than half of all families (Pandey, 1960; de Nettancourt, 2001; Allen & Hiscock, 2008; Rea & Nasrallah, 2008). By promoting xenogamy (outcrossing), SI effectively increases genetic variability within and among populations, thereby avoiding inbreeding depression and the consequent expression of deleterious recessive alleles. It has been suggested that the evolution of SI may represent one of the key innovations that enabled the rapid diversification of angiosperms during the Cretaceous (Whitehouse, 1950; Zavada & Taylor, 1986),

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although evolutionary inferences are complicated by the existence of several distinct types of SI, differing in genetic control and the site and timing of the incompatibility reaction (Allen & Hiscock, 2008). In most cases, incompatibility is pre-zygotic, manifested either as the failure of 'self pollen' to germinate on the stigma (sporophytic SI, in which the SI mechanism is under sporophytic genetic control) or the arrest of pollen tube growth in the stigma or style after germination of self pollen (gametophytic SI, under gametophytic genetic control). In some cases, however, the incompatibility reaction is post-zygotic (late-acting, or ovarian), in which the pollen tube developing from self pollen enters the ovary and gametic fusion occurs, leading to the initiation of fruit development. The incomplete formation of fruits following post-zygotic SI can be confused with inbreeding depression, although the abscission of immature fruits resulting from the latter is not restricted to a single stage, as observed in post-zygotic SI; the failure of fruit formation due to inbreeding depression is therefore expected to occur anytime during fruit-set, whereas post-zygotic SI operates at a particular time. The diversity and taxonomic distribution of these mechanisms suggests that SI is probably homoplasious in angiosperms (e.g. Charlesworth *et al.*, 2005).

Reconstruction of the evolution of SI in early-divergent angiosperms is further complicated by the prevalence of protogyny (the temporal separation of pistillate and staminate function in hermaphroditic flowers) and the occurrence of dioecy [e.g. *Amborella trichopoda* Baill. (Amborellaceae), in which floral unisexuality is inferred to be of recent evolutionary origin; Endress, 2001; Thien *et al.*, 2003]: the existence of either protogyny or dioecy would inevitably limit the selective advantage of SI. There are also conflicting interpretations of empirical data: *Illicium floridanum* J.Ellis (Illiciaceae), for example, has been variously interpreted as showing pre-zygotic SI (Thien, White & Yatsu, 1983) or either post-zygotic SI or inbreeding depression (Koehl *et al.*, 2004). Self-sterility of some form is reportedly widespread within the magnoliids (Canellales, Laurales, Magnoliales and Piperales; Allen & Hiscock, 2008), often with evidence of parthenocarpy (the development of fruits that lack functional seeds). The genetic basis of this incompatibility is often obscure, although there is convincing evidence of SI in Saururaceae (Pontieri & Sage, 1999), Trimeniaceae (Bernhardt *et al.*, 2003) and Winteraceae (Sage & Sampson, 2003), and rather more equivocal evidence in Chloranthaceae (Hristova *et al.*, 2005) and Illiciaceae (Thien *et al.*, 1983; Koehl *et al.*, 2004).

Annonaceae are one of the largest magnoliid families, with 112 genera and c. 2440 species (Couvreur *et al.*, 2011), and are of key importance because of the phylogenetic position of the magnoliids, sister either

to the eudicots (Soltis *et al.*, 2000; Zanis *et al.*, 2002) or (with Chloranthaceae) to a combined eudicot–monocot–*Ceratophyllum* clade (Jansen *et al.*, 2007; Moore *et al.*, 2007). Despite the accumulation of an extensive literature on the reproductive biology of Annonaceae, there is no convincing evidence of a biochemically mediated SI mechanism (Table 1) as the commonly reported failure to set fruit after self-pollination is likely to result merely from inbreeding depression.

There is unequivocal evidence of self-compatibility, with at least some fruit set after artificial self-pollination, in all the major phylogenetic lineages of the family (Table 1) including: subfamily Anaxagoreoideae (*Anaxagorea* A.St.-Hil.); subfamily Annonoideae tribes Annoneae (*Asimina* Adans.), Bocageae (*Cymbopetalum* Benth. and *Porcelia* Ruiz & Pav.), Duguetieae (*Duguetia* A.St.-Hil.), Guatterieae (*Guatteria* Ruiz & Pav.), Uvarieae (*Desmos* Lour. and *Uvaria* L.) and Xylopieae (*Xylopia* L.); and subfamily Malmeoideae tribe Miliuseae [*Hubera* Chaowasku, *Marsypopetalum* Scheff., *Mitrephora* Hook.f. & Thomson, *Monoon* Miq., *Pseuduvaria* Miq. and *Trivalvaria* (Miq.) Miq.] (subfamilial and tribal classification follows Chatrou *et al.*, 2012). The unequivocal absence of SI in so many disparate lineages, including the earliest diverging genus *Anaxagorea*, suggests that the family as a whole is probably genetically self-compatible. The maintenance of self-compatibility can have selective advantages as it ensures reproductive success under unfavourable conditions, including fragmented populations in which individuals are spatially isolated, populations that have undergone bottlenecks in size (experienced, for example, during the early establishment of populations), and when pollinator availability is limited.

Despite the apparent absence of SI mechanisms in Annonaceae and the potential advantages of retaining self-compatibility, it appears that outcrossing is common and dominates in many cases. Predominant outcrossing has been demonstrated, for example, in populations of *Asimina obovata* (Willd.) Nash and *A. pygmaea* (W.Bartram) Dunal (Norman & Clayton, 1986), *Maasia glauca* (Hassk.) Mols, P.J.A.Kessler & Rogstad and *M. hypoleuca* (Hook.f. & Thomson) Mols, P.J.A.Kessler & Rogstad [Rogstad, 1994; as '*Polyalthia glauca* (Hassk.) Boerl.' and '*Polyalthia hypoleuca* Hook.f. & Thomson', respectively], *Popowia pisocarpa* (Blume) Endl. (Momose, Nagamitsu & Inoue, 1998a), *Sapranthus palanga* R.E.Fr. (Bawa, 1974) and *Uvaria elmeri* Merr. (Nagamitsu & Inoue, 1997). Facultative outcrossing and mixed-mating systems (with limited self-fertilization) are also common in the family, observed in populations of *Asimina parviflora* (Michx.) Dunal (Norman, Rice & Cochran, 1992), *Hubera korinti* (Dunal) Chaowasku

Table 1. A review of fruit set after self-pollination and temporal dioecy in the Annonaceae, based on previous and current research

Species	Fruit set after self-pollination	Temporal dioecy	Reference(s)
<i>Anaxagorea crassipetala</i> Hemsl.	Yes	No	Armstrong & Marsh (1997); Bawa, Perry & Beach (1985)
<i>Anaxagorea dolichocarpa</i> Sprague & Sandwith	Yes	No	Braun & Gottsberger (2011)
<i>Annona cherimola</i> Mill.	Yes	Yes	Lora <i>et al.</i> (2011)
<i>Annona mucosa</i> Jacq. [as ' <i>Rollinia jimenezii</i> Saff. var. <i>nelsonii</i> R.E. Fr.']	?	Yes	Murray & Johnson (1987)
<i>Annona squamosa</i> L.	Limited	Yes	Wester (1910); Sahoo, Panda & Mohanty (2000)
<i>Asimina obovata</i> (Willd.) Nash	No	?	Norman & Clayton (1986)
<i>Asimina parviflora</i> (Michx.) Dunal	Limited	?	Norman <i>et al.</i> (1992)
<i>Asimina pulchella</i> (Small) Rehder & Dayton [as ' <i>Deeringothamnus pulchellus</i> Small']	Yes?	?	Norman (2003)
<i>Asimina pygmaea</i> (W. Bartram) Dunal	No	?	Norman & Clayton (1986)
<i>Asimina rugelii</i> B.L. Rob. [as ' <i>Deeringothamnus rugelii</i> (B.L. Rob.) Small']	Yes	?	Norman (2003)
<i>Cymbopetalum</i> Benth. sp.	Yes	?	Bawa <i>et al.</i> (1985)
<i>Dasyaschalon trichophorum</i> Merr.	?	Yes	Pers. observ.
<i>Desmos chinensis</i> Lour.	Yes	Yes	Pers. observ.
<i>Duguetia</i> A. St.-Hil. sp.	Yes	?	Gottsberger (1970)
<i>Guatteria</i> Ruiz & Pav. spp.	Yes	?	Gottsberger (1970)
<i>Hubera korinti</i> (Dunal) Chaowasku [as ' <i>Polyalthia korinti</i> (Dunal) Thwaites']	Limited	No	Ratnayake <i>et al.</i> (2006b)
<i>Maasia discolor</i> (Diels) Mols <i>et al.</i> [as ' <i>Polyalthia discolor</i> Diels']	?	No	Rogstad (1994)
<i>Maasia glauca</i> (Hassk.) Mols <i>et al.</i> [as ' <i>Polyalthia glauca</i> (Hassk.) F. Muell.']	No	No	Rogstad (1994)
<i>Maasia hypoleuca</i> (Hook. f. & Thomson) Mols <i>et al.</i> [as ' <i>Polyalthia hypoleuca</i> Hook. f. & Thomson']	No	Yes	Rogstad (1994)
<i>Maasia multinervis</i> (Diels) Mols <i>et al.</i> [as ' <i>Polyalthia multinervis</i> Diels']	?	No	Rogstad (1994)
<i>Maasia sumatrana</i> (Miq.) Mols <i>et al.</i> [as ' <i>Polyalthia sumatrana</i> (Miq.) Kurz']	?	Yes	Rogstad (1994)
<i>Marsypopetalum littorale</i> (Blume) B. Xue & R.M.K. Saunders [as ' <i>Polyalthia littoralis</i> (Blume) Boerl.']	Yes	?	Okada (1990)
<i>Mitrephora heyneana</i> (Hook. f. & Thomson) Thwaites	Limited	No	Weerasooriya & Saunders (2010)
<i>Monoon coffeoides</i> (Thwaites ex Hook. f. & Thomson) B. Xue & R.M.K. Saunders [as ' <i>Polyalthia coffeoides</i> (Thwaites ex Hook. f. & Thomson) Hook. f. & Thomson']	Limited	No	Ratnayake <i>et al.</i> (2006b)
<i>Popowia pisocarpa</i> (Blume) Endl.	No	?	Momose <i>et al.</i> (1998a)
<i>Porcelia goyazensis</i> R.E. Fr.	Yes	?	Gottsberger (1970)
<i>Pseuduvaria mulgraveana</i> Jessup	?	No	Pang <i>et al.</i> (2013)
<i>Sapranthus palanga</i> R.E. Fr.	No	?	Bawa (1974)
<i>Trivalvaria costata</i> (Hook. f. & Thomson) I.M. Turner	Yes	?	R. J. Wang, pers. comm.
<i>Uvaria cordata</i> (Dunal) Alston	Yes	No	Pang (2012)
<i>Uvaria elmeri</i> Merr.	No	?	Nagamitsu & Inoue (1997)
<i>Uvaria semecarpifolia</i> Hook. f. & Thomson	?	No	Attanayake (2010)
<i>Xylopiya brasiliensis</i> Spreng.	Limited	?	Andrade <i>et al.</i> (1996)
<i>Xylopiya championii</i> Hook. f. & Thomson	Limited	No	Ratnayake <i>et al.</i> (2007)

[Ratnayake *et al.*, 2006a, b, as '*Polyalthia korinti* (Dunal) Thwaites'], *Monoon coffeoides* (Thwaites ex Hook.f. & Thomson) B.Xue & R.M.K.Saunders (Ratnayake *et al.*, 2006a, b, as '*Polyalthia coffeoides* Hook.f. & Thomson'), *Xylopiya brasiliensis* Spreng. (Andrade, Oliveira-Filho & Soares, 1996) and *Xylopiya championii* Hook.f. & Thomson (Ratnayake *et al.*, 2007). There are no reports of obligate or predominant self-fertilization in Annonaceae, although *Cymbopetalum brasiliense* (Vell.) Benth. ex Baill. was recently inferred to be apomictic (Braun, Dötterl & Gottsberger, 2011).

Annonaceae display a broad array of strategies to promote xenogamy in the absence of SI mechanisms. Some of these strategies, such as protogyny, appear to be plesiomorphic and shared with other early-divergent angiosperms; some, such as floral unisexuality, appear to have evolved independently in Annonaceae; and others, such as disruptions to the reproductive phenology, appear to be unique. In this review, we aim to reassess the diversity of such mechanisms in a phylogenetic context, utilizing the extensively sampled, well-resolved and strongly supported molecular phylogenetic analyses of Annonaceae that have become available in recent years (e.g. Couvreur *et al.*, 2011; Chatrou *et al.*, 2012).

INTRAFLORAL DICHOGAMY

Intrafloral dichogamy, or the temporal separation of male and female sexual function in hermaphroditic flowers, is widespread in angiosperms and is an effective mechanism to avoid autogamy. In early-divergent angiosperms dichogamy is manifested as protogyny, in which pistillate function precedes staminate function (Gottsberger, Silberbauer-Gottsberger & Ehrendorfer, 1980; Lloyd & Webb, 1986; Bernhardt & Thien, 1987; Endress, 1990, 2001, 2010; Thien *et al.*, 2009). Of the 23 families of early-divergent angiosperms with at least some hermaphroditic flowers, 21 are protogynous (Endress, 2010), with the condition in the remaining two families, Gomortegaceae and Hernandiaceae, currently unknown. Protogyny is therefore probably the ancestral condition in angiosperms, and its presence is likely to have significantly limited the selective advantages favouring the evolution of alternative mechanisms to promote xenogamy.

All species of Annonaceae with hermaphroditic flowers are protogynous, although the degree of separation between the cessation of stigmatic receptivity and the onset of anther dehiscence is variable (Saunders, 2012). The receptive cycle typically lasts 24–48 h, extending over 2 or 3 days, with the pistillate and staminate phases separated by a non-receptive interim phase either overnight (in diurnal species; Fig. 1A) or during the day (in crepuscular or

nocturnal species; Fig. 1B). This effectively precludes autogamy, although geitonogamy is still possible if multiple sexually mature flowers of both functional phases occur simultaneously on the plant. The interim phase is brief in some species (e.g. 6 h in *Desmos chinensis* Lour.: our unpublished data), whereas in others the pistillate and staminate phases overlap, enabling possible autogamous self-pollination (e.g. *Uvaria concava* Teijsm. & Binn.: Silberbauer-Gottsberger, Gottsberger & Webber, 2003).

The ubiquitous occurrence of protogyny in hermaphroditic flowers in Annonaceae is likely to have imposed significant constraints on the evolution of alternative pollination systems. Unlike many plant families with equivalent levels of species diversity, Annonaceae are remarkably uniform in pollination biology (Saunders, 2012): the great majority of species are pollinated by beetles (with distinct small- and large-beetle pollination syndromes evident: Gottsberger, 1999, 2012; Silberbauer-Gottsberger *et al.*, 2003; Goodrich, 2012; Saunders, 2012), although thrips (Gottsberger, 1970; Webber & Gottsberger, 1995; Küchmeister *et al.*, 1998; Momose *et al.*, 1998a, b; Silberbauer-Gottsberger *et al.*, 2003) and flies (Gottsberger, 1985; Morawetz, 1988; Norman *et al.*, 1992; Su *et al.*, 2005) are also important pollinators to a lesser extent.

Unlike more derived angiosperms, bee pollination is comparatively rare in Annonaceae, and is only reported unequivocally in two genera: *Unonopsis* R.E.Fr. (Carvalho & Webber, 2000; Silberbauer-Gottsberger *et al.*, 2003; Teichert *et al.*, 2008) and *Uvaria* (Silberbauer-Gottsberger *et al.*, 2003). Saunders (2012) suggested that this rarity of bee pollination is probably due to the widespread occurrence of protogyny, as pollen-collecting bees would be unlikely to visit pistillate-phase flowers which lack a pollen reward (see also Endress, 2010). Silberbauer-Gottsberger *et al.* (2003) showed that *Uvaria concava* Teijsm. & Binn. is pollinated by meliponine bees (Apidae subfamily Meliponinae), which consume stigmatic exudate from pistillate-phase flowers and pollen from staminate-phase flowers; significantly, however, the authors also demonstrated temporal overlap between the pistillate and staminate phases. A similar mechanism has also been observed in *Uvaria grandiflora* Roxb. ex Hornem. (our unpublished data). Pollination by euglossine bees (Apidae subfamily Euglossini) has been demonstrated in two species of *Unonopsis*: *U. guatterrioides* R.E.Fr. (Carvalho & Webber, 2000; Silberbauer-Gottsberger *et al.*, 2003) and *U. stipitata* Diels (Teichert *et al.*, 2008). Although euglossine bees consume nectar, male bees also collect fragrant compounds from the flowers using hairs on their forelegs and subsequently use the scent to attract female bees (Williams, 1982). Bee

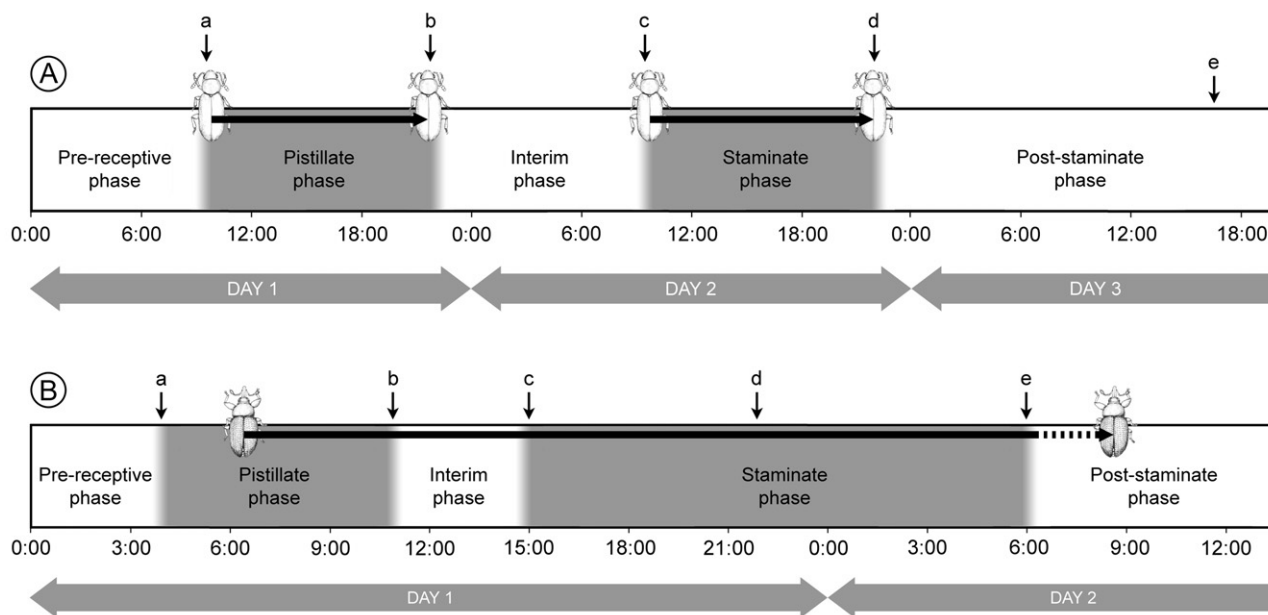


Figure 1. Flower-level phenology in selected species of Annonaceae. A, *Mitrephora heyneana* (redrawn from Weerasooriya & Saunders, 2010). B, *Dasymaschalon trichophorum* (previously unpublished data). Floral events indicated by letters: a, initial formation of stigmatic exudate, indicative of onset of stigmatic receptivity (both species), and opening of apertures in the corolla chamber (*D. trichophorum* only); b, drying of stigmatic exudate (both species); c, abscission of stigmatic head (*M. heyneana* only) and initiation of anther dehiscence (both species); d, closure of apertures in the corolla chamber (*D. trichophorum* only) and cessation of anther dehiscence (both species); e, abscission of petals (both species). Presence of beetle pollinators in the flowers indicated by horizontal black arrows (beetle drawings by Ngai Yuen Yi).

pollination in Annonaceae is therefore restricted to species in which protogyny has partially broken down (with a temporal overlap between the pistillate and staminate phases) or else species pollinated by bees that are attracted by rewards other than pollen.

HERKOGAMY

Herkogamy is the spatial separation of pollen presentation and receipt in a hermaphroditic plant (Webb & Lloyd, 1986; Richards, 1997). Herkogamic barriers in the form of inner staminodes, located between the fertile stamens and carpels, are common in some families in Magnoliales and Laurales. This form of herkogamy is rare in Annonaceae, although it is reported in the earliest diverging genus *Anaxagorea* (*A. javanica* Blume: Corner, 1940; Maas & Westra, 1984-85; *A. dolichocarpa* Sprague & Sandwith: Maas-van de Kamer, 1993; *A. brevipes* Benth.: Webber, 2002; and *A. prinoides* (Dunal) A. DC.: Teichert, Dötterl & Gottsberger, 2011). Although flowers of *Anaxagorea* are protogynous, the inner staminodes elongate and cover the stigmas towards the end of the pistillate phase, acting as a physical barrier to the transfer of pollen. The significance of this is obscure, however, in the absence of data on the temporal overlap of the pistillate and staminate phases.

Annona cherimola Mill. exhibits a different form of herkogamy (Lora, Herrero & Hormaza, 2011). The flowers are protogynous, although the periods of stigmatic receptivity are variable: autogamy is theoretically possible as the stigmas sometimes remain receptive after the start of the staminate phase, depending on environmental conditions. Lora *et al.* (2011) demonstrated the existence of herkogamy in *A. cherimola* flowers, in which the androecium and gynoecium are spatially separated by a barrier consisting of non-functional outermost carpels, adjacent to the stamens.

The species of *Anaxagorea* and *Annona* L. discussed above are pollinated by small beetles (Gazit, Galon & Podoler, 1982; Corner, 1988; Maas-van de Kamer, 1993; Webber, 2002). Such species generally adopt 'mess-and-soil' pollination (Fægri & van der Pijl, 1979), in which the beetles move around inside the floral chamber in search of food or the opportunity to mate. It is doubtful how efficient herkogamy is likely to be in self-compatible species adapted for 'mess-and-soil' pollination as the beetles can easily cross the spatial barrier between the androecium and gynoecium. Herkogamy is therefore more likely to play a significant role in flowers that are only weakly protogynous.

Uvaria buchholzii Engl. & Diels [Le Thomas, 1968, as '*Balanga buchholzii* (Engl. & Diels) Le Thomas'] and

all species of *Toussaintia* Boutique (Boutique, 1951; Deroin, 2000; Deroin & Luke, 2005) have an elongated receptacle, forming an androgynophore that may function to separate the stamens and carpels. There is unfortunately no empirical data on the pollination ecology of either taxon to assess the potential role of the androgynophore as a herkogamic barrier.

INTER- AND INTRA-INDIVIDUAL FLORAL SYNCHRONY

Although protogyny is an effective mechanism for avoiding autogamy, it does not prevent geitonogamy, in which pollen is transferred from staminate-phase to pistillate-phase flowers within the same individual. Floral synchrony within and between individuals in a population can be effective in preventing or reducing geitonogamy, however, and has evolved in many disparate lineages of self-compatible angiosperms (Endress, 2010). Two distinct types of inter- and intra-floral flowering synchrony can be discerned in Annonaceae: pistillate/staminate-phase floral synchrony and heterodichogamy.

PISTILLATE/STAMINATE-PHASE FLORAL SYNCHRONY

This is the most easily identified form of floral synchrony, in which pistillate-phase and staminate-phase flowers do not co-occur on an individual plant: all flowers on an individual mature in concert, entering the pistillate phase (and subsequently the staminate phase) simultaneously. As this form of floral synchrony occurs in combination with protogyny, both autogamous and geitonogamous self-pollination are prevented; two cohorts of individuals must therefore co-occur in the population to ensure cross-pollination. Floral organs, including stamens and petals, typically abscise soon after the end of the staminate phase, as prolonged retention of the stamens might result in temporal overlap with subsequent flowering events in the same individual, thereby enabling geitonogamy. The flowers of species exhibiting this form of floral synchrony typically have short anthesis periods, often over only 2 days with consecutive cohorts of flowers at different maturation stages generally separated by only one flowerless day (Endress, 2010).

Pistillate/staminate-phase floral synchrony is uncommon in early-divergent angiosperms, with previous reports from only *Eupomatia* R.Br. (Eupomatiaceae: Endress, 1984), *Canella* P.Browne (Canelaceae: Wilson, 1982) and three genera of Annonaceae, namely *Annona* (Murray & Johnson, 1987, as '*Rollinia* A.St.-Hil.'; Lora *et al.*, 2011), *Guatteria* (Webber, 2002) and *Maasia* Mols, Keßler & Rogstad (Rogstad, 1994, as '*Polyalthia* Blume'). It is

likely to have been overlooked in many genera and hence to be much more widespread, however: previously unpublished data on *Dasymaschalon trichophorum* Merr. and *Desmos chinensis* (Fig. 2A, B; Table 2), for example, demonstrate that these species also show such synchrony. Floral synchrony in these species is similar, with a flowering rhythm extending over *c.* 26 h (illustrated for *D. trichophorum* in Fig. 1B). A cohort of flowers matures on Day 1, entering the pistillate phase (a in Fig. 1B; and subsequently the staminate phase: c in Fig. 1B) simultaneously. Petals of the flowers in this cohort abscise in the early morning of Day 2 (e in Fig. 1B), encouraging the pollinators to depart. No flower buds mature on Day 2, resulting in a non-flowering day. The second cohort of flower buds matures on Day 3. The onset of flower bud maturation therefore occurs every second day with an intervening non-flowering day where no intra-individual transfer of pollen occurs. The flowering synchronies in *D. chinensis* and *D. trichophorum* do not operate strictly, however: occasional asynchrony occurs, in which a few flowers mature and enter the pistillate phase as the main cohort of flowers enters the staminate phase (indicated by asterisks in Table 2). Although few asynchronous flowers form relative to the number of flowers on the preceding day, geitonogamy can nevertheless occur. It might be advantageous for the population to retain a low level of geitonogamy, especially when population density is low (e.g. after adverse environmental conditions, or following the colonization of new areas). It is also likely that occasional irregularities in the pattern of floral synchrony will enable differential mixing of individuals in the two interbreeding groups, ensuring greater genetic variability in the population.

HETERODICHOGAMY

In some species floral synchrony is achieved through the co-occurrence of different floral morphs within populations. This is known as heterodichogamy, and has been reported in the early-divergent angiosperm orders Laurales and Magnoliales (Renner, 2001; Endress & Lorence, 2004), including Annonaceae: *Anaxagorea prinoides* (Teichert *et al.*, 2011) and *Annona squamosa* L. (Wester, 1910).

The most detailed study of heterodichogamy in Annonaceae is that of *Anaxagorea prinoides* by Teichert *et al.* (2011), on which the following discussion is based. Anthesis in flowers of *A. prinoides* (Fig. 2C) is considerably shorter than that observed in most species of Annonaceae (Fig. 3). Flowers of some individuals (designated 'morph A' and representing *c.* 50% of the population) enter the pistillate phase at *c.* 13:30 h and then transition to the staminate phase at



Figure 2. Floral diversity in selected species of Annonaceae discussed in the text. A, *Dasydaschalon trichophorum*; B, *Desmos chinensis*; C, *Anaxagorea prinoides*; D, *Pseuduvaria mulgraveana*; E, *Mitrephora heyneana*; F, *Xylopia championii*. A, B and D, © C.-C. Pang (D, reproduced from Pang *et al.*, (2013); C, © H. Teichert (reproduced from Teichert *et al.*, 2011 with permission from Springer-Verlag); E, F, © R. M. C. S. Ratnayake (F, reproduced from Ratnayake *et al.*, 2007 with permission from The University of Chicago Press).

c. 10:00 h of the next day. Another group of individuals ('morph B') enters the pistillate phase around 09:30 h, changing to the staminate phase around 13:00 h, with the end of the phase occurring about an hour later. This ensures that when flowers of one morph in the population are in their pistillate phase, flowers of the other morph will be in their staminate phase, and vice versa; this successfully promotes xenogamy as individual trees consistently represent only one morph. Individual trees produce many flowers simultaneously, with several of the trees monitored showing uninterrupted flowering. Transfer of pollen between flowers of morph B is unlikely given the temporal separation between receptive cycles. Although the temporal separation between flowers of morph A is considerably shorter and hence geitonogamy is possible, the timing of the cessation of the staminate phase in one morph and the initiation of the pistillate phase in the opposing morph are synchronized to encourage movement of the pollinating beetles between different morphs rather than flowers of the same morph.

UNISEXUAL FLOWERS

Most species of Annonaceae have solely hermaphroditic flowers, a condition that has been interpreted as ancestral for the family as a whole (Saunders, 2010). In many lineages of Annonaceae xenogamy has been promoted either by the evolutionary loss of the androecium or gynoecium in the flower, resulting in structurally unisexual flowers (dicliny), or else by alternative mechanisms that result in structurally hermaphroditic flowers that are functionally unisexual. Examples of the latter include incomplete pollen and/or stamen development and delayed anther dehiscence (equivalent to premature corolla abscission).

DICLINY

Dicliny, or the possession of unisexual flowers (either pistillate, staminate or both), has evolved in several disparate lineages in subfamily Annonoideae tribes

Table 2. Numbers of pistillate-phase flowers on individuals of *Dasymaschalon trichophorum* and *Desmos chinensis*, monitored over 10 consecutive days

Species; plant number	Day									
	1	2	3	4	5	6	7	8	9	10
<i>Dasymaschalon trichophorum</i>										
Plant 1	1	0	1	0	2	0	1	0	0	0
Plant 2	0	0	2	1*	2	0	1	0	2	0
Plant 3	0	0	0	0	0	7	0	7	0	2
Plant 4	2	0	2	0	6	0	3	0	4	0
Plant 5	0	0	0	0	0	4	0	1	0	0
Plant 6	0	0	0	0	0	0	2	0	4	0
<i>Desmos chinensis</i>										
Plant 1	0	2	0	4	0	0	0	0	0	0
Plant 2	9	0	10	0	8	1*	10	0	5	0
Plant 3	0	12	0	4	0	8	0	15	2*	8
Plant 4	3	0	7	1*	5	0	2	0	1	0
Plant 5	2	0	3	0	7	0	3	0	4	0
Plant 6	0	20	0	11	0	13	0	9	0	9
Plant 7	0	3	0	2	1*	2	0	1	0	2
Plant 8	1*	5	0	4	0	2	0	1	0	0
Plant 9	0	8	0	6	2*	1	0	8	0	3
Plant 10	0	0	0	0	37	0	15	0	7	0

Individuals generally show alternation between days in which pistillate-phase flowers are borne and days lacking such flowers; exceptions are indicated by an asterisk. Data are previously unpublished, based on observations from populations from Daoyin, Hainan, China (*D. trichophorum*), and Lung Fu Shan country park, Hong Kong (*D. chinensis*).

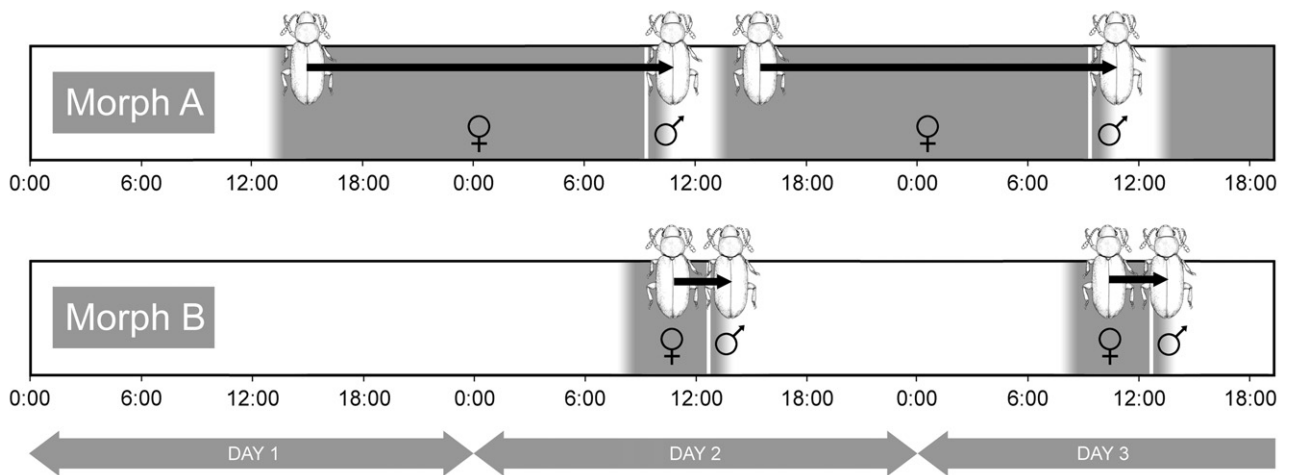


Figure 3. Flower-level phenology of two flowers on different individuals of *Anaxagorea prinooides* (redrawn from Teichert *et al.*, 2011), illustrating heterodichogamy. Flowers of ‘morph A’ (top row) have a receptive anthesis period extending from c. 13:30 h to c. 11:00 h the following day; flowers of ‘morph B’ (lower row) have a shorter period of receptivity, c. 09:30–14:00 h. Presence of beetle pollinators in the flowers indicated by horizontal black arrows (beetle drawings by Ngai Yuen Yi).

Annoneae (*Annona* spp. previously classified in *Raimondia* Saff.: Westra, 1995; *Anonidium* Engl. & Diels: Bakker, 2000; *Diclinanona* Diels: Bakker, 2000) and Monodoreae (*Uvariopsis* Engl.: Kenfack *et al.*, 2003)

and in subfamily Malmeoideae tribes Malmeae (*Ephedranthus* S.Moore: Oliveira & Sales, 1999; *Klarobelia* Chatrou: Chatrou, 1998; *Pseudephedranthus* Aristeg.: Oliveira & Sales, 1999; *Pseudomalmea*

Chatrou: Chatrou, 1998), Miliuseae [*Pseuduvaria*: Su & Saunders, 2006; *Stelechocarpus* (Blume) Hook.f. & Thomson: van Heusden, 1995] and Piptostigmatheae (*Greenwayodendron* Verdc.: Verdcourt, 1969; *Polyceratocarpus* Engl. & Diels: Bakker, 2000). In Annonaceae, the occurrence of unisexual flowers within and among individuals is occasionally manifested as monoecy, in which pistillate and staminate flowers co-occur on the same plant (e.g. *Pseuduvaria*: Pang *et al.*, 2013; *Uvariopsis*: Kenfack *et al.*, 2003). Dioecy, in which pistillate and staminate flowers are borne on separate individuals, has been reported in *Pseuduvaria* (Silberbauer-Gottsberger *et al.*, 2003; Su & Saunders, 2006), although experimental field data is required to verify this. In the majority of Annonaceae species with unisexual flowers, however, unisexuality either occurs as andromonoecy, in which staminate and hermaphroditic flowers co-occur on the same plant, or androdioecy, in which staminate and hermaphroditic flowers are borne on different individuals. Distinguishing between andromonoecy and androdioecy is difficult in the absence of field-based studies of living populations, as herbarium collections typically consist of isolated branches with only a limited number of flowers.

The best-documented example of a genus of Annonaceae with structurally unisexual flowers is *Pseuduvaria*, in which the majority of species have been inferred to have unisexual flowers (Su & Saunders, 2006). Su, Smith & Saunders (2008) inferred that floral unisexuality was ancestral in *Pseuduvaria*, although the genus itself was shown to be derived from ancestors with hermaphroditic flowers. Pang *et al.* (2013), however, showed that the staminate function in hermaphroditic flowers in *Pseuduvaria mulgraveana* Jessup is limited but not entirely lost, contradicting previous assumptions (Su & Saunders, 2006; Su *et al.*, 2008) that the 'staminodes' in hermaphroditic flowers are sterile; this suggests that sex expression in the genus needs to be reappraised.

There is evidence in *Pseuduvaria* of evolutionary reversal to fully hermaphroditic flowers in species that are endemic to New Guinea [*P. becarrii* (Scheffer) J.Sinclair, *P. brachyantha* Y.C.F.Su & R.M.K.Saunders and *P. nova-guineensis* J.Sinclair: Su *et al.*, 2008]. It is speculated that this may have increased potential for self-pollination, which may have provided a selective advantage during the colonization of new regions due to the enhanced ability to establish from a small number of initial individuals. This would be particularly beneficial in geographical areas such as New Guinea, which are topographically and ecologically complex, with extensive mountain ranges that exceed altitudinal habitat preferences in the genus (Su & Saunders, 2009).

Darwin (1877) recognized two types of floral unisexuality, distinguishing between flowers that possess a remnant of the non-functional sexual organ (i.e. those that have become unisexual as a result of selective organ abortion) and those in which no relictual organ is apparent (i.e. those that are unisexual from inception). This typological approach was criticized by Mitchell & Diggle (2005), who recommended an alternative scheme, which distinguishes between the origins of unisexual flowers from hermaphroditic ancestors as a result of: (1) selective organ abortion, with the retention of a remnant of the non-functional organ; (2) an extension of this scenario, in which the relictual organ is lost entirely; and (3) the evolution of unisexual flowers from inception. The widespread occurrence of sterile staminodes in functionally pistillate flowers of *Pseuduvaria* suggests that in this genus, at least, the first two of these scenarios are relevant (Su *et al.*, 2008).

INCOMPLETE POLLEN AND STAMEN DEVELOPMENT

As discussed above, several genera in Annonaceae (including *Pseuduvaria*, which has been well documented: Su & Saunders, 2006; Su *et al.*, 2008; Pang *et al.*, 2013) exhibit andromonoecy or androdioecy, with a combination of staminate and hermaphroditic flowers. It has been hypothesized that the structurally hermaphroditic flowers in such species may be functionally pistillate due to the formation of sterile staminodes that either lack pollen or produce sterile pollen grains (Charlesworth, 1984; Saunders, 2010), rendering the populations functionally monoecious or dioecious. Su & Saunders (2006) reported incomplete pollen development in structurally hermaphroditic flowers of *Pseuduvaria macrocarpa* (Burck) Y.C.F.Su & R.M.K.Saunders: although no pollen germination tests were performed, pollen from structurally hermaphroditic flowers was inferred to be sterile as it is smaller (equatorial axis *c.* 20 µm) than pollen from staminate flowers (*c.* 25 µm) (Su & Saunders, 2006). The retention of sterile pollen grains in hermaphroditic flowers is likely to serve as a food reward for the pollen-consuming pollinators (Saunders, 2010), although empirical studies are required to corroborate this and to determine whether *P. macrocarpa* is structurally androdioecious or andromonoecious.

Considerable caution is required before assumptions of stamen and pollen functionality are accepted, however. Empirical data on the reproductive biology of *Pseuduvaria mulgraveana* (Fig. 2D; Pang *et al.*, 2013), for example, contradict the previously held assumption that the species has functionally unisexual flowers, with pistillate flowers with sterile staminodes (Su & Saunders, 2006). Pang *et al.* (2013) demonstrated that the species is andromonoecious,

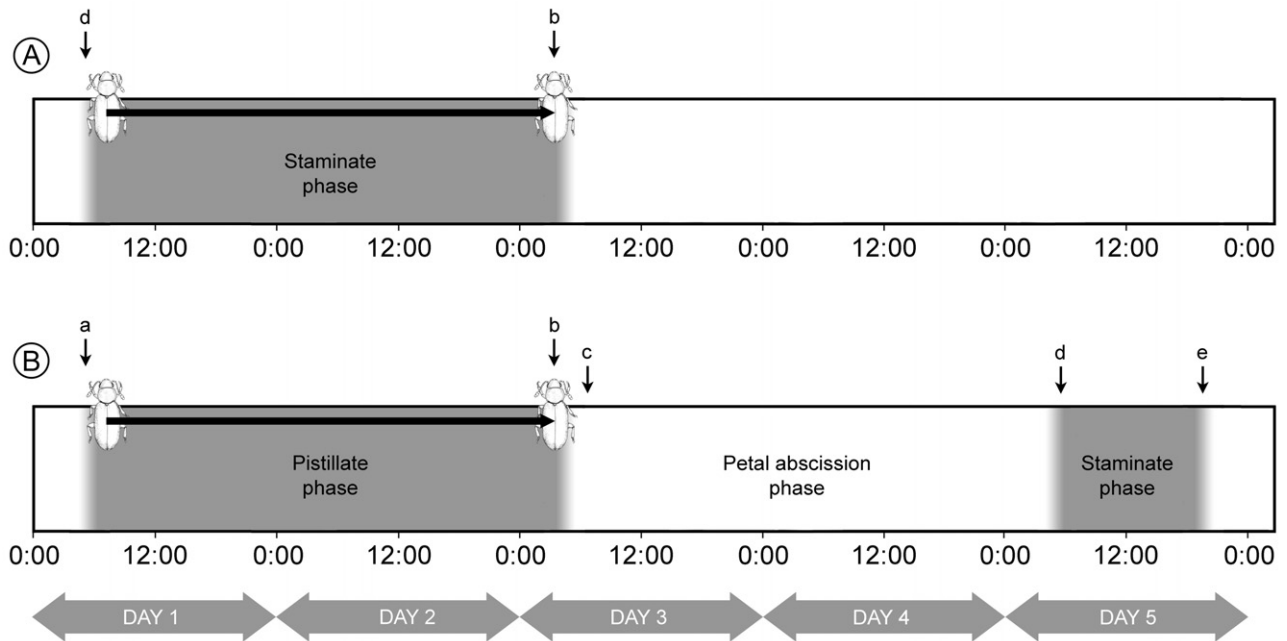


Figure 4. Flower-level phenology of staminate (A) and hermaphroditic (B) flowers of *Pseuduvaria mulgraveana* (redrawn from Pang *et al.*, 2013). Floral events indicated by letters: a, initial formation of stigmatic exudate, indicative of stigmatic receptivity; b, petal abscission; c, cessation of stigmatic receptivity; d, initiation of anther dehiscence; e, abscission of stamens. Presence of beetle pollinators in the flowers indicated by horizontal black arrows (beetle drawings by Ngai Yuen Yi).

and that the flowers previously assumed to be functionally pistillate are actually hermaphroditic, albeit with relatively few stamens (*c.* 10).

DELAYED ANTHOR DEHISCENCE

Other mechanisms exist in Annonaceae to achieve separation of floral sex in addition to the evolution of dicliny and the loss of pollen viability in structurally hermaphroditic flowers. As discussed above, Pang *et al.* (2013) have demonstrated that *Pseuduvaria mulgraveana* (Fig. 2D) is andromonoecious, with distinct staminate and hermaphroditic flowers, both of which produce viable pollen. The staminate flowers are receptive for *c.* 2 days (Fig. 4A), with petal abscission occurring in the early morning of the third day (b in Fig. 4A). As with all hermaphroditic flowers in the family, those of *P. mulgraveana* are protogynous (Fig. 4). The pistillate phase is prolonged over 2 days, ending around 05:00 h on the second day of receptivity (c in Fig. 4B). The mitriform inner petal dome abscises from the flower slightly before the end of the pistillate phase (b in Fig. 4B). As the petals are presumably the major attractant for the pollinators, emitting floral odour, offering visual cues and providing a shelter or tryst site, the beetle pollinators leave the flowers after petal abscission. Dehiscence of the anthers is delayed by 2–5 days (d in Fig. 4B), and

although the pollen is viable there are no beetles present and so pollen transfer to another flower is not possible. The structurally hermaphroditic flowers of *P. mulgraveana* are therefore functionally pistillate, despite possessing fertile pollen.

The delayed anther dehiscence (or premature corolla abscission) described for *P. mulgraveana* has not been reported in any other species in Annonaceae, and as far as we are aware is unique among angiosperms. As autogamy is avoided by protogyny, which is ubiquitous in Annonaceae, delayed anther dehiscence presumably assists in preventing geitonogamy as fewer staminate flowers are available and the number of mature flowers is limited at any one time within an individual of *Pseuduvaria mulgraveana* (Pang *et al.*, 2013).

PETAL MOVEMENT

Most species of Annonaceae possess floral chambers that are derived from either the inner whorl of petals or both whorls of petals collectively. The chambers are generally only weakly enclosed, resulting from the loose coherence of adjacent petals: pollinators are therefore free to enter or leave the flower at any stage, and the chambers generally do not act as true pollinator ‘traps’. Even in species in which the inner

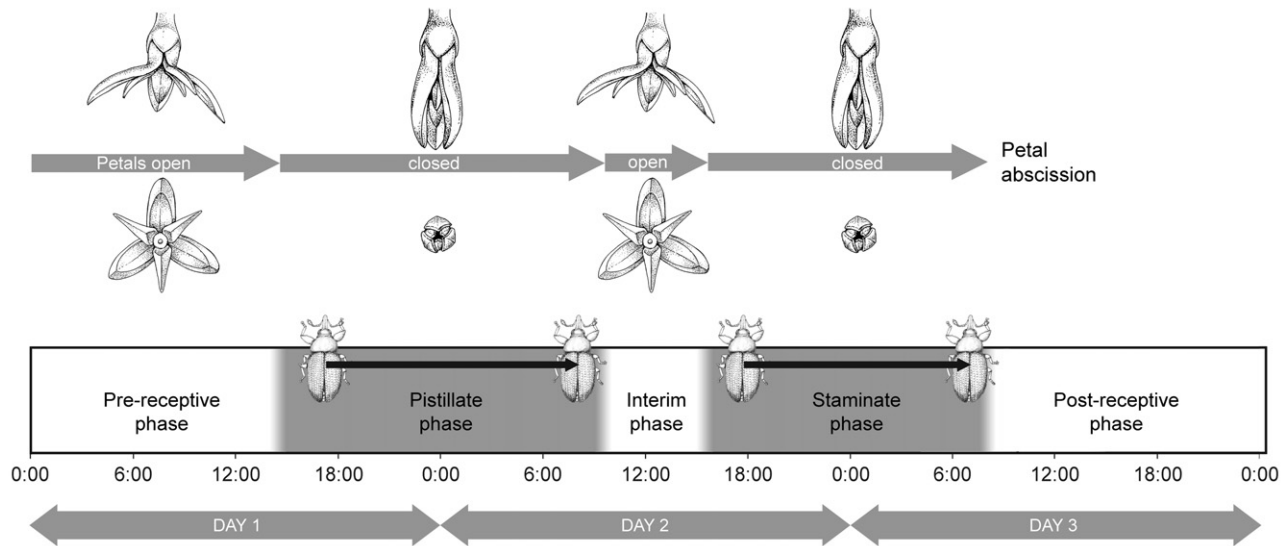


Figure 5. Flower-level phenology of *Xylopia championii* (data derived from Ratnayake *et al.*, 2007). Presence of beetle pollinators in the flowers indicated by horizontal black arrows (beetle and flower drawings by Ngai Yuen Yi).

petals are apically connivent, forming a coherent mitriform dome that abscises from the flower as a single unit at the end of anthesis, apertures are maintained between adjacent petals, enabling movement of pollinators. The extreme structural diversity of floral chambers reflects extensive homoplasy, with multiple independent evolutionary origins in the family (Saunders, 2010). The chambers presumably function by protecting pollinators from predators and adverse fluctuations in environmental conditions and providing a possible tryst site. The chambers may also function by creating a micro-environment for the maintenance of elevated temperatures resulting from thermogenesis.

In most cases, the floral chamber forms at the onset of the pistillate phase, and remains partially closed throughout the period of floral receptivity until the end of the staminate phase, when the corolla abscises. The pollinators (most commonly beetles) remain in the flower throughout anthesis, carrying pollen from staminate-phase flowers as they depart in search of pistillate-phase flowers. Species of Annonaceae that exhibit this pattern typically have short receptive periods of *c.* 24–48 h, with the onset of the pistillate phase synchronized with the end of the staminate phase in other flowers (Fig. 1B) so that the pollen-laden beetles are therefore able to move directly from flower to flower. It is likely that the beetles that pollinate such species have a unimodal pattern of daily activity, with the start and end of the receptive phases aligned with the peak period of beetle activity.

Other species of Annonaceae appear to be aligned with bimodal patterns of daily activity in beetles (e.g.

crepuscular beetles, which are active at dusk and dawn), and exhibit a correspondingly longer period of receptivity. *Mitrephora heyneana* (Hook.f. & Thomson) Thwaites, for example, has large apertures between the apically connivent inner petals (Fig. 2E), which always remain open, allowing the beetle pollinators to arrive or leave the flower at any stage. Empirical data on the pollination ecology of this species (Weerasooriya & Saunders, 2010) have revealed that the beetles visit the flowers at the onset of the pistillate phase (*c.* 09:00–10:00 h) and gradually begin to leave as stigmatic receptivity ceases (from *c.* 20:00 h) (Fig. 1A). Another cohort of beetles subsequently arrives as the flower enters its staminate phase (*c.* 09:00–10:00 h on the following day) and again gradually begins to leave from *c.* 20:00 h. Each flower therefore receives two cohorts of beetles, corresponding with the bimodal cycle of daily activity shown by the beetles.

In other species that are adapted for pollination by beetles with a bimodal pattern of activity, the floral chamber is sufficiently tightly closed to partially impede beetle movement; in these cases the petals separate during the non-receptive interim phase between the pistillate and staminate phases, enabling arrival and departure of beetles. This is observed in *Xylopia championii* (Figs 2F, 5; Ratnayake *et al.*, 2007), *Hubera korinti* (Ratnayake *et al.*, 2006a, as '*Polyalthia korinti*') and *Monoon coffeoides* (Ratnayake *et al.*, 2006a, as '*Polyalthia coffeoides*').

Although these petal movements do not directly promote xenogamy, there is a clear correlation with floral synchrony. Species adapted for pollination by

beetles that show a unimodal pattern of daily activity rely on beetle movement from flower to flower at the same time each day: pollen-laden beetles are attracted to pistillate-phase flowers soon after departing from late- or post-staminate flowers. This would significantly increase the potential for geitonogamous self-pollination unless floral synchrony is operating. In the species for which experimental field data have been collected, there is clear evidence for such a correlation (e.g. *Dasymaschalon trichophorum*: Fig. 1B; Table 2). In marked contrast, species adapted for pollination by beetles with a bimodal pattern of daily activity (e.g. *Mitrephora heyneana*: Fig. 1A; and *Xylopiya championii*: Fig. 5) have less need for floral synchrony, as the beetles departing from the flowers (at the end of either the pistillate or the staminate phases) must survive outside the floral chamber for much longer, including two periods of activity; it is suggested that this increases opportunity for movement between flowers that are spatially more distant, lessening the chances of geitonogamy.

As noted above, the flowers of *Xylopiya championii* (Figs 2F, 5), *Hubera korinti* and *Monoon coffeoides* are unusual among species of Annonaceae as the floral chamber opens during the non-receptive interim phase (Ratnayake *et al.*, 2006a, 2007). Saunders (2010, 2012) speculated that the beetles may be attracted to the flowers by sexual deceit (although evidence for sex pheromone mimicry in the floral scents is rather equivocal), and that they may be less likely to move to other flowers after mating; a fresh cohort of unmated beetles might therefore be more effective in dispersing pollen. It is also possible, however, that the changes in petal orientation are simply influenced by environmental factors, such as light and temperature, that fluctuate according to a daily rhythm.

DISCUSSION

Phylogenetic reconstructions of early-divergent angiosperms have consistently retrieved a sister-group relationship between Annonaceae and Eupomatiaceae (e.g. Doyle & Endress, 2000; Soltis *et al.*, 2000; Zanis *et al.*, 2002), with a more distant relationship with the other magnolialean families (Degeneriaceae, Himantandraceae, Magnoliaceae and Myristicaceae). With the exception of Myristicaceae (which are sister to the other families in the order), Magnoliales are dominated by species with hermaphroditic, protogynous flowers. These characteristics are likely to be ancestral for Annonaceae (Saunders, 2010), occurring in all major lineages in the family, including the earliest diverging genus *Anaxagorea* (Maas & Westra, 1984–85). Hermaphroditic flowers are also apparent in the Late Cretaceous fossil *Futabanthus asami-*

gawensis Takahashi *et al.*, which is hypothesized to have a phylogenetic position at the crown of the Annonaceae clade (Takahashi *et al.*, 2008).

Although biochemically mediated SI mechanisms have evolved independently in several disparate angiosperm lineages, they do not occur in Annonaceae. Despite the prevalence of self-compatible hermaphroditic flowers in the family, reproduction is predominantly or exclusively xenogamous, with autogamous and geitonogamous self-pollination avoided in various ways. Some of the mechanisms that promote xenogamy in Annonaceae probably pre-date the origin of the family; their presence is likely to significantly constrain the selective advantage of SI, and hence may explain the retention of self-compatibility in the family.

The widespread occurrence of protogyny amongst early-divergent angiosperms clearly reflects its efficacy in avoiding autogamy. This presumably provides some explanation for the comparative rarity of herkogamy in Annonaceae, as the spatial separation of pistillate and staminate function would only provide a selective advantage when protogyny has partially or fully broken down. It is significant that *Annona cherimola*, which is reportedly herkogamic due to the separation of the androecium and gynoecium by a barrier consisting of sterile outer carpels, exhibits a breakdown in protogyny, with some overlap between the cessation of stigmatic receptivity and the onset of anther dehiscence (Lora *et al.*, 2011). Herkogamy has also been reported in *A. prinoides*: although this species does not show overlap between the pistillate and staminate phases, the former phase transitions directly into the latter without a non-receptive interim phase (Teichert *et al.*, 2011).

Although protogyny is likely to be effective in preventing autogamy, it can only assist in preventing geitonogamy in combination with other mechanisms. Many species of Annonaceae bear relatively few sexually mature flowers concurrently on an individual, inevitably reducing opportunities for geitonogamy; it is likely, however, that many other factors influence the number of concurrent flowers, including resource constraints on fruit number. Floral synchrony is effective at preventing geitonogamy in combination with protogyny, and has been reported from several phylogenetically disparate lineages of the family. Pistillate/staminate-phase floral synchrony, in which pistillate-phase and staminate-phase flowers are not borne concurrently on an individual, has primarily been reported from subfamily Annonoideae, occurring in tribes Annoneae (*Annona*: Murray & Johnson, 1987, as '*Rollinia*'; Lora *et al.*, 2011), Guatterieae (*Guatteria*: Webber, 2002) and Uvarieae [*Dasymaschalon* (Hook.f. & Thomson) Dalla Torre & Harms and *Desmos*: previously unpublished data], but has

also been reported from subfamily Malmeeoideae tribe Maasieae (*Maasia*: Rogstad, 1994, as '*Polyalthia*'). Heterodichogamy, in which two phenologically distinct and reproductively isolated morphs co-occur in a population, has been reported from the earliest diverging subfamily Anaxagoreoideae (*Anaxagorea*: Teichert *et al.*, 2011) and the more derived subfamily Annonoideae tribe Annoneae (*Annona*: Wester, 1910). Given the presence of floral synchrony in the sister lineage *Eupomatia* (Eupomatiaceae; Endress, 1984), we hypothesize that floral synchrony may be an ancestral condition in Annonaceae as a whole, although unequivocal interpretation would require much more extensive field observations. Since heterodichogamy is considerably more complex than pistillate/staminate-phase synchrony, it seems reasonable to assume that it represents a more specialized and derived form of floral synchrony. Despite the inadequacy of available data, we suggest that heterodichogamy is likely to have evolved independently in *Anaxagorea* and *Annona*, although future investigations may reveal it to be more common in the family. Different floral phenological patterns can be identified in Annonaceae, correlated with daily cycles of activity in beetles, which are the dominant pollinator guild in the family. Plant species pollinated by beetles that exhibit a unimodal daily periodicity in activity (i.e. with peak activity only once during the day) typically have the timing of the pistillate and staminate phases synchronized so that the departure of pollen-laden beetles from late-staminate-phase flowers occurs at the same time as the onset of the pistillate phase in other flowers. Species with this phenological pattern are likely to show floral synchrony. In contrast, plant species that are pollinated by beetles that display a bimodal pattern of daily activity (e.g. crepuscular beetles, which are active at dusk and at dawn) do not show any synchrony between the end of the staminate phase and the onset of the pistillate phase; the beetles are required to seek shelter outside the floral chamber for several hours, increasing opportunities for movement between flowers on different individuals.

The widespread occurrence of protogyny in Annonaceae (with or without associated floral synchrony) has constrained the evolution of unisexual flowers as there is no clear selective advantage for the latter in terms of promoting xenogamy. Monoecy is accordingly rare in the family, and there are no unequivocal reports of dioecy that are substantiated by field data. Floral unisexuality is usually manifested as andromonoecy (or possibly androdioecy), in which staminate and hermaphroditic flowers coexist in populations. The selective advantage of andromonoecy may be related to the increased pollen production per individual rather than directly promoting xenogamy, par-

ticularly in cases where pollinator limitation may be significant. It has also been suggested, however, that the evolution of andromonoecy may be related to the high cost of fruit and seed production, as it inevitably increases pollen competition among individuals, thereby increasing reproductive fitness (Spalik, 1991; de Jong, Shmida & Thuijisman, 2008). In some cases, however, populations that are structurally andromonoecious may be functionally monoecious, with retention of stamens in the structurally hermaphroditic but functionally pistillate flowers as a pollen reward for floral visitors. This appears to be the case in *Pseuduvaria macrocarpa*, as a result of incomplete pollen development (Su & Saunders, 2006), and *Pseuduvaria mulgraveana*, as a result of delayed anther dehiscence (Pang *et al.*, 2013).

Recent research into sexual function in *Pseuduvaria* (Pang *et al.*, 2013) has highlighted the caution that is required in assessments of floral sexual function: interpretations based solely on herbarium collection can be misleading and result in erroneous deductions. Experimental field-based studies are required to assess the breeding system of a plant species, including detailed monitoring of floral phenology.

CONCLUSIONS

Most species in the early-divergent angiosperm family Annonaceae possess structurally hermaphroditic flowers. Despite lacking a biochemically mediated SI mechanism, the great majority of Annonaceae species are predominantly or exclusively xenogamous. Xenogamy is promoted in diverse ways, some of which are common in early-divergent angiosperms, others which have evolved independently and others which appear to be unique to the family.

Protogyny, in which pistillate function precedes staminate function within flowers, is uniformly present in all families of Magnoliales (except Myricaceae, which have unisexual flowers) and in almost all other magnoliid families, and occurs in all species of Annonaceae with hermaphroditic flowers. Protogyny is effective at preventing autogamy (self-pollination involving pollen transfer within a single flower), but has constrained evolutionary shifts in pollination system: pollination by pollen-collecting bees is rare in the family, as such bees are unlikely to visit pistillate-phase flowers which offer no pollen reward.

Herkogamy, or the spatial separation of pollen presentation and receipt within a flower, has only been reported in two genera in Annonaceae. It is unlikely to significantly promote xenogamy in the family as most species are adapted for 'mess-and-soil' beetle pollination, in which beetles can easily cross the

herkogamic barrier. The selective advantage of herkogamy in Annonaceae would also be limited by the efficacy of protogyny.

Although protogyny is effective in preventing autogamy, it cannot prevent geitonogamy (self-pollination involving pollen transfer between different flowers on the same individual). Two different forms of floral synchrony have evolved in the family to avoid geitonogamy. Pistillate/staminate-phase floral synchrony, in which pistillate-phase and staminate-phase flowers do not co-occur on an individual, is hypothesized to be the ancestral condition in Annonaceae. Heterodichogamy, in which two phenologically distinct and reproductively isolated morphs co-occur in populations, is hypothesized to be derived, and to have evolved independently in at least two lineages in the family. Floral synchrony is only reported in species in which the end of the staminate phase in the flower coincides with the onset of the pistillate phase on other flowers; these species are adapted for pollination by beetles with a unimodal rhythm of daily activity.

Structurally unisexual flowers are rare in Annonaceae as protogyny constrains the selective advantage in terms of promoting xenogamy. There are no unequivocal reports of dioecy in the family. Floral unisexuality is usually manifested in the family as andromonoecy, in which staminate and hermaphroditic flowers coexist. Putative advantages of this include increased pollen production correlated with pollinator limitation and increased reproductive fitness due to greater pollen competition.

Structural andromonoecy may represent an alternative route to full monoecy, however, through loss of staminate function in structurally hermaphroditic flowers. Examples of this in Annonaceae include incomplete pollen and/or stamen development in structurally hermaphroditic flowers and delayed anther dehiscence, in which the corolla abscises (resulting in premature departure of the pollinators) prior to pollen release.

Floral phenology is complex in dichogamous species and can play a key role in promoting xenogamy. It is imperative that assessments of plant breeding system incorporate such studies.

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