5 Reproductive Biology

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5.1 Introduction

Most studies on the reproductive biology of species of the genus *Clusia* are limited in time, space and phenological scales that they cover. Floral biology descriptions are more abundant than studies on the possible ecological and evolutionary causes and consequences of sexual behaviour. For instance, Table 5.1, which is largely based on a previous list by Lopes and Machado (1998), shows information on 28 species more thoroughly studied, which adds up to ca. 10 % or less of the 250–400 species belonging to the genus (Bittrich and Amaral 1996a; Pipoly et al. 1998).

Despite these limitations, it is now well established that the reproductive biology of the genus *Clusia* often comprises three features which, individually, are relatively rare among angiosperms: dioecy, resin as reward for pollinators and floral mimetism. A fourth feature, equally rare, is agamospermy, the occurrence of which in the genus is still highly controversial. However, it is not always that all four features appear jointly for a given species. The various possible cases of single or combined occurrences of these features in a given species turns the reproductive biology of *Clusia* a very challenging subject, which might provide new insights into the ecology and evolution of sexual behaviour in plants.

These features began to emerge already in the earliest papers on *Clusia* reproductive behaviour. Janzen (1971) described the interaction of *Clusia* with resin-collecting bees, such as the Euglossini, but did not report on resin collection. Skutch (1971) first reported *Clusia* pollination by bees that collected secretion of the stamens, which prompted a series of studies examining the role of resin. The first report on agamospermy for the genus has been provided by Maguire (1976), who studied monoecious populations of *C. rosea*. However, agamospermy in *Clusia* is much less covered in the literature than the role of resin in pollination, perhaps because it now appears to be less com-

Table 5.1. Some of the main findings regarding aspects of the reproductive biology of *Clusia* species and respective sources

Species	Reward			Vector[a]	AM/MP	Agamospermy	RS	Comments	References
	Pollen	Nectar	Resin						
C. alata Pl. et Tr.	?	No	√	Mainly *Trigona corvina* (Cockerell 1913)	?	?	D	–	Ramirez and Gómez (1978)
C. aemygdioi Gomes da Silva et Weinberg	√	No	No	Mainly Trigonini and Halictidae	√	No	D	Ongoing research	G. Matallana – personal communication (Fig. 5.1E,F)
C. arrudae Pl. et Tr.	√	No	√	*Eufriesea* spp. *Melipona quadrifasciata* (Lepeletier 1836), *Trigona spinipes* (Fabricius 1793)	?	No	D	–	Carmo and Franceschinelli (2002)
C. burle-marxii Bittrich	√	No	No	Beetles	?	?	?	–	V. Bittrich apud Lopes and Machado (1998)
C. columnaris Engl.	√	No	√	Mainly Trigonini and Meliponini	?	?	D	–	Bittrich and Amaral (1996a)
C. criuva Camb.	√	No	No	Beetles	√	No	D	–	Correia et al. (1993)
C. fluminensis Pl. et Tr.	√	No	√	*Euglossa cordata* (Linnaeus 1758), *Plebeia mosquito* (Smith 1863), *Tetragonisca angustula* (Latreille 1811), and Trigonini	?	No	D	Pollen and resin mixture forms fruits, while pollen alone does not	V. Bittrich apud Carmo and Franceschinelli (2002), Correia (1983), Correia et al. (1989)
C. grandiflora Splitg.	√	No	√	Mainly Euglossini, but Trigonini also visit to collect pollen	?	?	D	Pollen in droplets of an oily liquid; differences in antibacterial resin effect by the genders	Bittrich and Amaral (1997), Lokvam and Braddock (1999)

Species				Pollinators			D/H	Notes	References
C. gundlachii Stahl.	?	No	✓	–	✓	?	D	–	Gustafsson (2000)
C. hilariana Schlecht.	✓	No	✓	Mainly Halictinae	✓	?	D	High pollen viability	Cruz et al. (1990), Faria et al. (2006), our own data
C. insignis Sem.	✓	No	✓	Mainly Euglossini	✓	?	D	–	Bittrich and Amaral (1997)
C. intermedia Mariz	?	✓	No	Flies	?	No	D	Ongoing research	G. Matallana – personal communication (Fig. 5.1A,B)
C. lanceolata Camb.	✓	No	✓	Mainly T. spinipes, and E. cordata	?	No	D	–	Correia et al. (1999)
C. leprantha Mart.	✓	No	✓	Trigonini	?	?	D	Pollen and resin mixture	Bittrich and Amaral (1997)
C. marizii Gomes da Silva	✓	No	✓		?	No	D	Small bees as pollinators	G. Matallana – personal communication (Fig. 5.1C,D)
C. minor L.	?	?	?	–	?	No	H	–	Hammel (1986), Bittrich (1994)
C. nemorosa G. Mey	✓	No	✓	Mainly Meliponini, and Trigonini	?	No	D/H	Maguire (1976) found indirect evidence of agamospermy	Maguire (1976); Bittrich and Amaral (1997); Lopes and Machado (1998)
C. odorata Sem.	?	No	?	Trigonini	?	?	D	First description of resin as reward	Croat (1978); Ramírez and Gómez (1978); Armbruster (1984)
C. organensis Pl. et Tr.	?	?	?	–	?	?	D	High pollen viability	Cruz et al. (1990)
C. pana-panari (Obley) Choisy	?	No	✓	Mainly Trigonini	?	?	D	Pollen and resin mixture; thick staminodes; male flowers may present pistillode	Armbruster (1984); Bittrich and Amaral (1997)

Table 5.1. (*Continued*)

Species	Reward			Vector[a]	AM/MP	Agamospermy	RS	Comments	References
	Pollen	Nectar	Resin						
C. paralicola G. Mariz	?	?	√	*T. spinipes*	?	?	?	–	V. Bittrich apud Lopes and Machado (1998)
C. parviflora Engl. nom. illeg.	√	?	–	Mainly beetles and flies	?	?	?	Pollinated by beetles and flies	V. Bittrich apud Lopes and Machado (1998)
C. pusilla Steyerm.	√	No	–	*Euglossa* spp., Halictidae, and *Xilocopa* spp.	√	?	D	Mainly pollinated by small bees	Bittrich and Amaral (1996a)
C. renggerioides Pl. et Tr.	?	No	√	*Trigona* spp.	?	?	D	Pollen and resin mixture; apolar fluid on the stigmas	Bittrich and Amaral (1997)
C. rosea Jacq.	?	No	√	*Euglossa*	?	√	M	Indirect evidence of agamospermy	Maguire (1976)
C. schomburgkiana (Pl. *et* Tr.) Benth. ex Engl.	?	No	√	*Duckeola ghilianii* (Spinola 1853) and *Trigona* spp.	?	?	D/H	Hermaphroditism was found by Maguire (1959) and not found by Bittrich and Amaral (1996a)	Maguire (1959) apud Bittrich and Amaral (1996a)
C. aff. *spathulaefolia* Pl. *et* Tr..	?	?	?	Beetles	?	?	?	–	V. Bittrich apud Lopes and Machado (1998)
C. scrobiculata Benoist	?	?	?	–	?	?	H	Briefly mentioned in this reference	Bittrich and Amaral (1996a)

[a] Please note that the years following the name of authorities are in agreement with rules of zoological nomenclature, which differs in that sense from botanical rules. Therefore, these cannot be found in the list of references

AM/MP=automimicry/mistake pollination; RS=reproductive system; H=hermaphrodite; D=dioecious; M=monoecious; ?=information unavailable for the given species

mon among *Clusia* species than initially expected (Lopes and Machado 1998; Correia et al. 1999; Carmo and Franceschinelli 2002).

This chapter reviews studies on the reproductive biology of *Clusia* and focuses particularly on the four main reproductive features of the genus: dioecy, resin as reward for pollinators, floral mimetism and the debate around agamospermy and vegetative reproduction. Finally, we use *C. hilariana* as a case study to discuss how such background might benefit, in the future, from a more integrated approach linking molecular biology and ecophysiology to population and community ecology.

5.2 Main Reproductive Features

5.2.1 Dioecy

Hermaphroditism is the dominant condition in flowering plants and dioecy is estimated to occur in only ca. 6 % of the entire angiosperm flora (Renner and Ricklefs 1995). Dioecious plants demand vectors for cross-pollination, and out-crossing avoids the consequences of inbreeding depression (Charlesworth and Charlesworth 1978; Thomson and Barrett 1981; Lloyd 1982; Sakai et al. 1995a; Freeman et al. 1997). Dioecy has been correlated with plant spatial distribution, tropical floras, oceanic islands and oligotrophic environments (Freeman et al. 1976; Bawa 1980a; Flores and Schemske 1984; Bawa et al. 1985; Sakai et al. 1995a, b; Thompson and Edwards 2001; Matallana et al. 2005), and since Darwin (1877) it has been suggested that resource allocation is a causal factor in the evolution and maintenance of dioecy.

Dioecy is the most common condition in the tribe Clusieae Choisy to which *Clusia* belongs. Thus, most *Clusia* species are dioecious and hermaphroditism (as in *C. scrobiculata*) is rare (Bittrich and Amaral 1996a). However, hermaphroditic flowers have been reported in occasional populations of predominantly dioecious species. Maguire (1966) was the first to report the occasional occurrence of hermaphroditic flowers in many scandent or epiphytic species. For instance, populations with dioecious and gynodioecious plants were found for *C. minor* (Maguire 1976), *C. nemorosa* (Mesquita and Franciscon 1995; Bittrich and Amaral 1997; Lopes and Machado 1998) and *C. schomburgkiana* (Bittrich and Amaral 1996a, 1997). In addition, Lopes and Machado (1998) found in *C. nemorosa* flowers of hermaphroditic individuals with a number of stamens smaller than average. These authors considered this as a type of 'female inconstancy'. Lloyd (1976) coined this term, which he defined as the presence of stamens in pistillate flowers. This peculiarity has been described for some species of *Clusia* by Maguire and Wurdack (1961), Maguire (1966, 1978) and Mariz (1974).

Dioecy requires cross-pollination. There are three classes of pollinator reward in *Clusia*: resin (which is probably the most common, if species number is considered), pollen and nectar, which are discussed next.

5.2.2 Resin

Resin is a rare floral reward among angiosperms. It occurs as a resource in *Dalechampia* (Euphorbiaceae; Armbruster 1984; Armbruster and Webster 1979), *Chrysochlamys, Tovomitopsis, Clusia* (tribe Clusieae at Clusiaceae; Gustafsson et al. 2002), *Clusiella* (Clusiaceae; Bittrich and Amaral 1996a, b), *Moronobea* (Clusiaceae; Vicentini and Fischer 1999) and also *Monstera* (Araceae), which produces a viscous stigmatic exudate (Ramírez and Gómez 1978). Early investigations focused on the roles of resin, primarily the *Dalechampia*-bees relationship (Armbruster and Webster 1979, 1981, 1982; Armbruster and Herzig 1984; Armbruster 1986; Armbruster and Steiner 1992; Armbruster et al. 1992). It has been claimed that resin provides adaptive advantages regarding attractiveness to bees, given that many such insects use resin for building nests (Armbruster 1984; Armbruster and Webster 1979; Bittrich and Amaral 1996a, b) and as a sticky defense against ant attack (Sakagami et al. 1989). Thus, resin attracts specific visitors, unlike pollen and nectar (Armbruster 1984). The use of resin as a food resource is suggested by Ramirez and Gomes (1978), but Armbruster (1984) argues that this hypothesis is improbable due to the toxicity, low nutritive constitution and resistance to decay and digestion. Furthermore, it is a predictable resource in time and space, since it takes longer than pollen and nectar to be entirely removed by visitors, and maintains viscosity for a long time (Armbruster 1984), conserving fungicidal and bactericidal properties (Lokvam and Braddock 1999).

Euglossini and some smaller bees are probably the main pollinators for *Clusia*. These typical forest bees cover large distances (up to 24 km) to collect resin, often in a traplining behaviour (Armbruster 1984; Janzen 1971; Roubik 1992; Lopes and Machado 1998). Thus, Janzen (1971) highlighted that

Fig. 5.1A–H. Flowers and pollinators of *Clusia*. A–F are sympatric plants at Santa Lúcia Biological Reserve, Santa Teresa municipality, state of Espírito Santo, SE-Brazil. **A** Diptera visiting a male flower of *C. intermedia* on a nectar based pollination system (see Table 5.1). **B** Female flower of *C. intermedia* visited by *Trigona spinipes* (Hymenoptera, Apidae). **C** Male flower of *C. marizii* visited by *T. spinipes*. **D** Female flower of *C. marizii*. **E** Male flower of *C. aemygdioi* visited by *T. spinipes*. **F** Female flower of *C. aemygdioi* visited by Halictinae in searching behaviour. **G** Male flower of *C. hilariana* **H** Female flower of *C. hilariana*. Photographs C–F were kindly provided by Glória Matallana, and G,H were kindly provided by Izar Aximoff
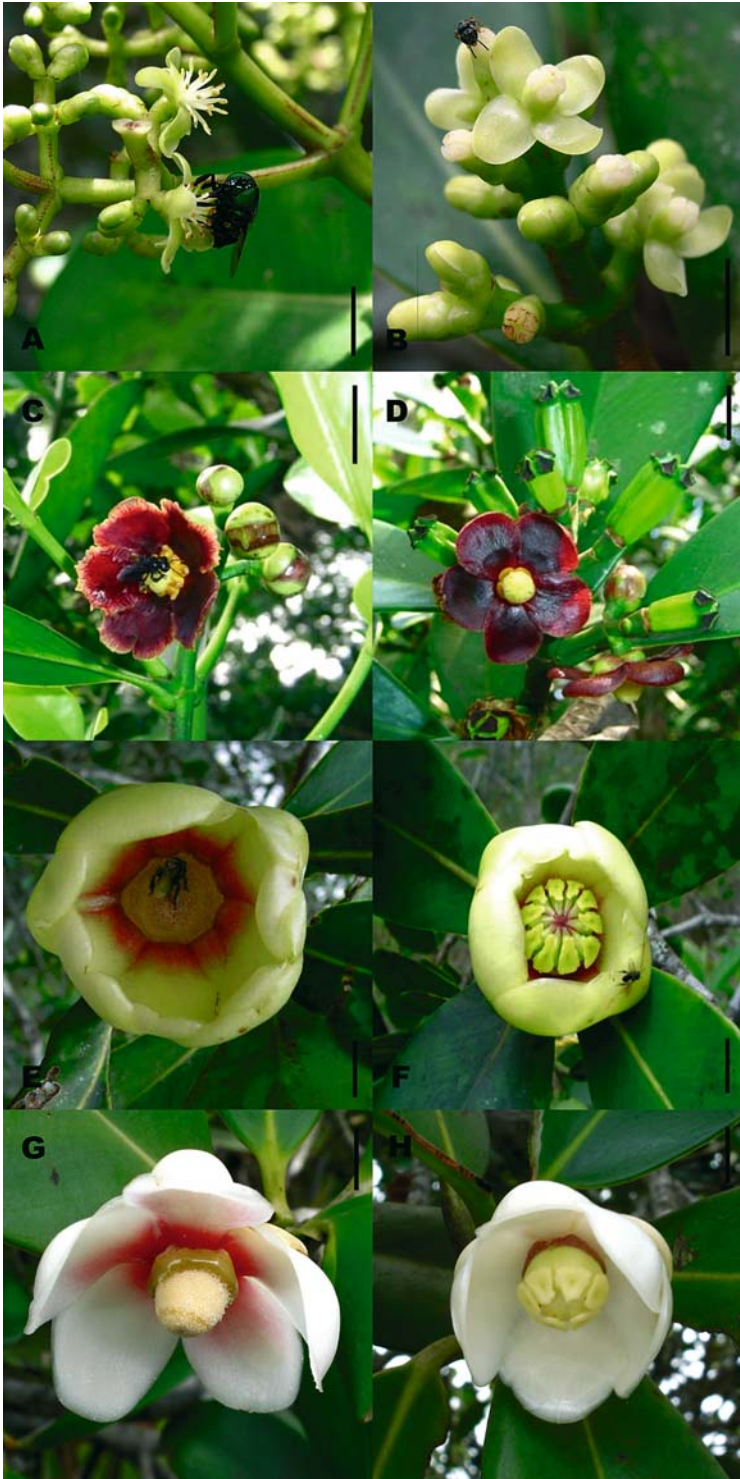
Euglossini is an important pollinator for plants that occur at low densities. These are the main pollinators of *C. arrudae* (Carmo and Franceschinelli 2002), *C. grandiflora* (Armbruster 1984, Bittrich and Amaral 1997), *C. rosea* (Armbruster 1984), *C. nemorosa* (Mesquita and Franciscon 1995; Bittrich and Amaral 1997; Lopes and Machado 1998), *C. insignis, C. leprantha, C. renggerioides, C. pana-panari* (Bittrich and Amaral 1997), *C. pusilla* (Lopes and Machado 1998) and *C. lanceolata* (Correia et al. 1989).

According to Bittrich and Amaral (1996a), flowers from different species have different traits to minimize the contact between resin and pollen when bees collect resin (Fig. 5.1). Thus, the infrageneric groups of *Clusia* with species offering resin, show a high diversity of forms. For further discussion on the evolution of resin-secretion see Armbruster (1984), Bittrich and Amaral (1996a, 1997), Gustafsson et al. (2002), Gustafsson and Bittrich (2003) and Chap. 6.

5.2.3 Automimetism and Mistake Pollination

Reproductive mimicry in plants is the utilization of false sensory cues to attract pollinators (Wiens 1978). One type of floral mimetism is the so-called automimicry syndrome, henceforth automimetism, where female flowers mimic male flowers that are the ones carrying food reward (Little 1983). Automimetism was first examined in more details by Baker (1976), while studying *Carica papaya* L.

In *Clusia*, automimetism is unmistakably recognized only in the case of pollen flowers. Female flowers do not produce pollen and in order to be pollinated they must look, or at least smell like the pollen-producing male ones (e.g., *C. criuva* and *C. gundlachii*; Correia et al. 1993; Gustafsson 2000). However, for some *Clusia* species with pollen flowers, resin and/or nectar are also produced by both sexes. In such cases, when pollen is the main reward tracked by pollinators, the visits in female flowers are described as mistake pollination (e.g., *C. insignis* and *C. pusilla*; Bittrich and Amaral 1996a, 1997). This poses a doubt as to whether mistake pollination is induced by automimetism or by a lack of specificity for pollen reward by the pollinator. For instance, Bawa (1980b) proposed that automimetic female flowers must offer rewards to the pollinators regardless of the degree of resemblance with male flowers, particularly when grouped in patches and spatially separated from males. This might apply to the *Clusia* cases discussed here.

Automimicry was first registered for the genus in *C. criuva* (Correia et al. 1993), which has pollen flowers pollinated by beetles. However, unlike *C. criuva*, all *Clusia* species reported to have mistake pollination are pollinated by small bees. While Euglossini are clearly important pollinating bees when resin is the floral reward (see Sect. 5.2.2), small bees are described as effective pollinators of some *Clusia* species (Lopes and Machado 1998). These pollina-

tors regularly collect floral resin and pollen (Ramírez and Gómez 1978; Correia 1983; Correia et al. 1989, 1999) but in some *Clusia*, small bees collect only pollen as in *C. pusilla* pollinated by Halictinae (Bittrich and Amaral 1996a), and *C. fluminensis* pollinated mainly by *Plebeia mosquito* (Lopes and Machado 1998).

Clusia pusilla provided the first study in *Clusia* to consider differences on floral output between male and female plants (Bittrich and Amaral 1996a). This species conformed to the model proposed by Bawa (1980b), where automimetic plant populations often have abundant male flowers, in excess over mimetic female flowers, and a long floral display for both genders. This difference in floral output between genders might be promoted by competition between male plants; however the different genders may, on the other hand, bear the same total amounts of reward. The smaller number of female, mimetic flowers may result in a higher number of visits in these flowers without reward.

Clusia nemorosa, pollinated by the small bee *Trigona spinipes*, also has mimetic female flowers (Lopes and Machado 1998). Bawa and Opler (1975) argue that smaller bees are particularly effective pollinators when distances between male and female are small, since small bees will not fly long distances. Considering the ability of some *Clusia* species to colonize open areas and form large populations, pollination by small bees can be more common than described to date. One would then expect that automimicry in dioecious plants would be more common for large, dense populations at open areas. As expected, *C. nemorosa* forms large populations in open areas in the restingas of northeast Brazil. Automimicry in plants pollinated by small bees, such as most *Clusia* species, would then probably have evolved when bee deception by floral mimicry is facilitated by the small distances between male and female plants that are more likely to occur in dense populations, as will be discussed in Sect. 5.3 dedicated to *C. hilariana*.

5.2.4 Asexual Reproduction: Agamospermy and Vegetative Propagation

The term agamospermy refers to the production of fruits and seeds by non-fertilised flowers. It is a type of asexual reproduction that combines the possible advantages of perpetuating a well-succeeded genotype through time, with the advantages of the higher mobility of seeds as propagules, unlike other forms of asexual reproduction such as vegetative propagation (Crawley 1997; Martins and Oliveira 2003). In *Clusia* literature and elsewhere, agamospermy is often referred to as 'apomixis'. However, apomixis is more precisely defined as any type of asexual reproduction, either through agasmospermy or vegetative reproduction (Kearns and Inouye 1993; Harris and Harris 2001). Therefore, here we will use agamospermy whenever we refer to seed production without fertilisation.

There has been some controversy around the occurrence and the relevance of agamospermy for *Clusia*'s reproduction (Mesquita and Franciscon 1995). For instance, Correia (1983) reported agamospermy for *C. fluminensis*, in flowers that received resin in the stigmas. Since there were doubts about the purity (i.e., absence of pollen) of the resin, new tests were conducted by Correia et al. (1989) comparing *C. fluminensis*, *C. lanceolata* and *C. criuva*. They confirmed the occurrence of agamospermy triggered by resin for *C. fluminensis* and also for *C. lanceolata*. However, the same research group found no fruit formation by agamospermy for *C. lanceolata* ten years later (Correia et al. 1999). Instead, experimental pollination by resin and pollen mixed, showed the highest fruit production among all treatments undertaken. This phenomenon had already been previously described by Bittrich and Amaral (1996a) to occur in *C. renggerioides* and *C. pana-panari* who found that more-or-less apolar fluids secreted by stigmas probably serves to dilute resin drops mixed with pollen. In the case of *C. fluminensis*, Carmo and Franceschinelli (2002) refer to unpublished data of V. Bittrich that indicated that the application of filtered resin in stigmas resulted in no fruit production. Therefore, it would appear that so far undisputed agamospermy in *Clusia* only exists in *C. rosea* (Maguire 1976) and *C. minor* (Maguire 1976; Hammel 1986).

Vegetative propagation and clonal growth, however, have been less investigated. Some field evidences have been found for some of the restinga species of *Clusia*, (e.g., *C. hilariana*; Correia 1998; Scarano et al. 2004). This type of reproduction is a common feature to many other restinga shrubs (Cirne and Scarano 2001). In the next section we discuss the possible relevance of this type of reproduction to population maintenance and growth of *C. hilariana*.

5.3 The Case of *Clusia hilariana*

We have pinpointed *C. hilariana* for a case study, not only for the obvious fact that it has been our focal species for a number of years (Scarano 2002; Scarano et al. 2005), but mostly because our data set allows for the examination of the species' reproductive biology from a molecular perspective all the way to a plant community perspective. Reproductive biology studies for *Clusia* species have often focused on floral biology, as reviewed above, and time and spatial scales covered hardly ever permit a deeper ecological understanding of causes and consequences of *Clusia*'s sexual behaviour.

Our interest in the reproductive biology and population ecology of *C. hilariana* started shortly after we found evidence for the important ecological role played by this species in the restingas of northern Rio de Janeiro state (particularly at the Restinga de Jurubatiba National Park - RJNP), Brazil: it is the most abundant tree species in the open restingas and acts as a nurse plant to various other species (see Chap. 4).

One of the earliest studies was a M.Sc. Dissertation carried out by Correia (1998), who found 312 *Clusia* plants in 0.5 ha of the RJNP. They were divided in three age classes, based on diameter size: young (32 plants, i.e. ca. 10 % of the total; those less than 20 cm in height), juveniles (211 plants) and adults (69 plants; those with a basal diameter =3.5 cm). This population structure suggests that regeneration and recruitment is not continuous for this species, which had more adults than seedlings. This was unexpected for a plant that is so abundant locally. Perhaps more interestingly, Correia could determine of the plants, which are less than 50 cm in height (n=59), those which had originated from seeds and those which originated from vegetative propagation. She found that 72 % of all plants less than 50 cm tall originated from seeds, where 2 % had germinated on the ground and 70 % within the tanks of terrestrial bromeliads (mainly *Aechmea nudicaulis* (L.) Griseb. and *Neoregelia cruenta* (Graham) L.B.Sm.; see also Scarano 2002). Forming adventitious roots the seedlings grow out from the tanks as they age and establish themselves as independent trees. The remaining 28 % of the young plants originated from vegetative propagation sprouting out from roots of mature trees.

These data, collected during one growing season only, indicated that abundant regeneration could be a periodic phenomenon (i.e., dependent on "good years") and that success apparently depended on tank bromeliads, which nurse seed-originated young plants (this pattern has also been found for *C. fluminensis* in the restinga of Barra de Maricá, further south; Macêdo and Monteiro 1987; Zaluar 2002; see also Chap. 4), and on the potential to reproduce vegetatively (see also Scarano et al. 2004).

We then decided to investigate this issue from a pollination biology perspective. *Clusia hilariana* is highly abundant locally. Antonovics and Levin (1980) proposed that plants at high densities are visited by pollinators more frequently than those at low densities (see also Larson and Barrett 2000). Therefore, one could expect high fruit and seed set locally. In contrast to this prediction, but in harmony with the findings of Correia (1998), we found a low production of fruit and viable seeds in natural open-pollinated as compared to hand-pollinated flowers (Faria et al. 2006). Plants commonly mature fewer fruits and seeds than could be produced given flower and ovule numbers (for reviews see Burd 1994; Larson and Barrett 2000). However, fruit set in our control plants was considered low in comparison to Sutherland and Delph (1984). They found a mean value for fruit set for 31 different dioecious species surveyed of 52.6 %. Conversely, the values we found for open-pollinated *C. hilariana* for two reproductive seasons were ca. half that average and ranged from 22 % to 26 %. Other studies on dioecious *Clusia* species further confirmed that the fruit set values obtained for open-pollinated *C. hilariana* were indeed low: *C. criuva*, a forest tree, showed 90 % fruit set in open- and hand-pollinated plants (Correia et al. 1993), and *C. nemorosa* in restingas showed 33.9 % fruit set in open-pollinated plants vs 86.1 % in hand-pollinated treatment (Lopes and Machado 1998).

We then attributed this low success to pollinator scarcity. The fact that there is no seed production via agamospermy in this species and that seed germination in situ is apparently almost restricted to the interior of bromeliad rosettes (Scarano 2002, Scarano et al. 2004) makes the high abundance of *C. hilariana* all the more puzzling.

Thus, the results of Correia (1998) and Faria et al. (2006) combined led us to two alternative explanations for the local persistence and dominance of *C. hilariana* despite low levels of fruit and seed set. 1) Successful years may occur irregularly and at long time intervals, such that longer-term studies are necessary to ensure sampling of such years. Low levels of reproduction sustained over time might be enough to ensure population stability. 2) Persistence and abundance may have been achieved by asexual reproduction.

At this point, it was clear that we needed to enhance both spatial and temporal scales of observations to verify whether any or both of the two alternatives above could explain *C. hilariana*'s high abundance in the RJNP. The study of Correia (1998) covered only one reproductive season and was conducted in a plot of 0.5 ha, while Faria et al. (2006) covered two seasons in a plot of 2 ha. Understanding the mechanisms of reproductive biology which ensure the high abundance of *C. hilariana* in this site seemed essential to foster conservation and management initiatives (Barbosa et al. 2004), since this very abundance seems to be responsible for much of the biodiversity and the functioning of the restinga ecosystem (Dias et al. 2005, 2006). Therefore, in order to deal with this issue, we decided to monitor two additional reproductive seasons and combine three approaches. 1) We increased the detail of pollinator observation and also of floral features to assess automimicry. 2) We increased the number of areas to observe fruit and seed formation. Since previous studies have described the existence of variation in terms of percent ground covered by this open restinga vegetation (low, intermediate and high; Pimentel 2002; Sampaio et al. 2005), we monitored aspects of reproductive biology in nine locations, each with a different vegetation cover. 3) In each of these locations we took leaf samples for DNA analysis and assessment of genetic diversity. These studies are the subject of an ongoing doctoral thesis (R.L. Martins, in preparation), but we point out below some of the main results. A large scale study of population structure and dynamics is also being carried out, but we still have no data available to discuss here.

5.3.1 Automimicry in *Clusia hilariana*

Clusia hilariana partly fits the pattern of automimicry proposed by Little (1983): (a) mimetic female flowers have features resembling male ones; (b) there is a higher floral output of male flowers than mimetic female flowers per area per day; (c) pollinators make shorter visits to the mimetic female flowers than to male flowers; and (d) male flowers produce pollinator reward (a high pollen

production) and females do not (Fig. 5.1G). Additionally, the mimetic female flowers have a high stigmatic surface (Fig. 5.1H), which increases the chance of pollen arrival and fecundation. The only aspect that does not clearly fit Little's model is the fact that mimetic female flowers produce resin. This may imply that probably two alternative pollinating systems might co-occur. First, and mainly, a system driven by automimicry, where small Halictinae bees are the main pollinators of *C. hilariana*, aiming for pollen as reward. Second, and probably less effective, a system where Euglossini are the pollinators aiming for resin as a reward that is more abundantly available in male than in female flowers.

5.3.2 Effects of Population Spatial Distribution in Fruit Set

In order to test as to whether spatial distribution of males and females was somehow related to individual fruit set, we established circular plots of 35 m radius around each of 53 flowering females, within 9 sampling locations. For each plot we measured: (a) distance from each female to the nearest flowering male; (b) number of flowers of the nearest male; and (c) number of male and female flowers within the whole plot. We found that fruit set in *C. hilariana* was strongly related to the proximity of flowering female plants to flowering males (t=−2.09; df=49; P<0.041); however only 14 % of the fruit production might be explained by multiple correlation ($F_{3,49}$=2.85; P<0.05; r^2=0.14; Y=16.9–0.31 $X_{a)}$+0.02 $X_{b)}$−1.92 $X_{c)}$). This is in harmony with the prediction of Bawa and Opler (1975) who proposed, based on body size and visiting behaviour, that smaller bees (such as the Halictinae) increase the chances of pollen flow when plant population is dense.

5.3.3 The Effects of Local Vegetation Cover on Fruit Set

The data discussed in Sect. 5.3.2 suggest an effect of the spatial arrangement of *C. hilariana* on its reproduction success. The findings of Pimentel (2002), indicating variation in terms of percent ground covered by the open restinga vegetation of the RJNP (low, intermediate and high; see also Sampaio et al. 2005), allowed us to investigate further the interference of spatial variation on reproductive success by analysing the correlations between vegetation cover and fruit set for plants of *C. hilariana*. We found considerable variation in fruit set of *C. hilariana* among nine locations, each with a different vegetation cover (Table 5.2). Further, we compared the patterns for two consecutive years. Fruit set was correlated to vegetation cover, however, while correlation was positive in 2004 (F=7.83; P<0.05; R^2=0.52), it turned out to be negative in 2005 (F=5.76; P<0.05; R^2=0.45). This seems to be related to the fact that while 7 out of 9 locations had a reduction in fruit set (more drastic in some than in others), 2 locations (10 and 11), conversely, had an increase in fruit set in 2005

Table 5.2. Percent vegetation cover (VC) of each of nine locations (numeration follows Pimentel 2002 and Sampaio et al. 2005) at the open restinga formation of RJNP, and respective fruit set (%) per location. Values in parenthesis are number of flowers assessed

Location	VC	2004	2005
1	34.6	27.4 (58)	16.2 (69)
2	33.0	30.4 (49)	17.8 (73)
3	38.2	45.7 (49)	19.4 (62)
4	56.4	46.0 (58)	10.0 (60)
5	28.5	13.3 (48)	11.6 (69)
6	29.5	24.4 (56)	17.7 (62)
8	37.0	28.7 (50)	15.9 (63)
10	20.0	27.0 (50)	33.3 (55)
11	28.0	15.2 (49)	32.2 (60)

despite lower rainfall. This dramatic difference in behaviour between years is puzzling, very difficult to interpret at this point, and obviously calls for further data collection. It is beyond the scope of this review to explore this matter any deeper, but it suffices to our goals in this chapter to highlight two points that emerge from this preliminary data: 1) spatial variation in fruit set is very high; and 2) vegetation cover alone does not explain this variation.

5.3.4 Population Genetics

Despite the scarcity of reports on the development, characterization and use of microsatellite loci in tropical plant species, Hale et al. (2002) had already characterized 13 polymorphic loci developed from *C. minor* (5) and *C. nemorosa* (8) and tested the transferability to 17 different *Clusia* species and assessed the degree of polymorphism only to *C. minor* and *C. ducu* Benth. Here, we tested the transferability of these same microsatellite markers to *C. hilariana* and assessed the degree of polymorphism for the amplified loci.

We collected leaves of 38 *C. hilariana* plants at the nine different locations described above at RJNP in order to perform microsatellite (SSR) analysis aiming to evaluate genetic diversity at the population level. DNA was extracted from leaves dried with silica gel by a modified CTAB method (Ferreira and Grattapaglia 1998). For all experiments, loci amplifications were performed as described by Margis et al. (2002) and sized by comparison to a 30–300-bp AFLP DNA ladder (Invitrogen).

Eight out of 13 primers pairs tested amplified loci of *C. hilariana*. This was possibly due to genome sequence homology, more specifically by the conservation of microsatellite flanking regions among closely related species (Dayanadan et al. 1997; White and Powell 1997). *Clusia hilariana* amplified

Table 5.3. Polymorphism information for *Clusia hilariana* and two other *Clusia* species studied by Hale et al. (2002) using microsatellite loci isolated from *C. minor* (Clm) and *C. nemorosa* (Cln) – = no amplification. Hale et al. (2002) data can be assessed in GenBank by numbers AY095353 to AY095365. Note that all products of amplification using primers from Clm for *C. hilariana* have a size range smaller than that found for the other 17 species, while those using primers from Cln fit on the same size range

Locus	Repeat in clone	Primer (5' to 3')	Hale et al. (2002) Size range (bp) for 17 *Clusia* species	No. of alleles C. minor (n=5)	C. ducu (n=5)	This study Size range (bp) for C. hilariana	No. of alleles C. hilariana (n=38)
Clm1	[TC]$_{28}$	F: TCCAAAGGTATGCATCAGAGG R: GCAGAAACATGCACTCACAAA	166–262	6	6	–	–
Clm2	[AG]$_5$AA[AG]$_{13}$	F: GATCAACGTGCAGGAGCTAA R: CACTGTTTGCACAATCTTGCT	172–200	4	2	82–85	2
Clm3	[CT]$_{11}$GTC[AC]$_9$	F: CCTCTCTCTCCCACCTCCTT R: TTGGCTTCTTTCTTTTGCAG	99–151	4	–	–	–
Clm4	[TC]$_4$TAC[TC]$_{10}$	F: AAGTTTTGGACCCGATTCGT R: GGCGATGTCATCACATTTG	169–241	1 (5)	2	98	1
Clm5	[GT]$_{10}$	F: ACAATGGTCCTGCTCTGAGG R: CAAGAGCTGCTATCCCTGCTA	185–195	3	2	70	1
Cln1	[GA]$_{10}$[GA]$_4$	F: AGGCTGAGAGTTCCAACACG R: TCTTACCTTGAAGAATCTTTTCCTT	211–279	–	–	–	–
Cln2	[TC]$_{18}$	F: TGGTTGACCCCTCTAACAGTG R: GCAAACAACTCAATCAGAATGC	211–249	4	3	260–270	3

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Table 5.3. (*Continued*)

Locus	Repeat in clone	Primer (5' to 3')	Hale et al. (2002) Size range (bp) for 17 Clusia species	No. of alleles C. minor (n=5)	C. ducu (n=5)	This study Size range (bp) for C. hilariana	No. of alleles C. hilariana (n=38)
Cln3	[TC]$_6$TT[TC]$_{11}$	F: AGCAATGGTGAACCCGATAA R: GATCTTTATGTCAAACCAAATATACTG	136–168	3	4	137–145	2
Cln5	[AG]$_{29}$A$_4$[AG]$_5$	F: ATTGAACAAGCTGGGCACTC R: TCACCTTGGGCCTTTCTCTA	128–255	2	2	–	–
Cln6	[TA]$_{14}$T$_8$[AC]$_9$[AT]$_7$	F: GATCTCGGGTCAACCAACA R: TGATGCTACAATATAAACGAATGG	165–239	2	2	180	1
Cln7	[TC]$_{13}$	F: CTTACGCCGAGGTTGAAGTC R: GCTCACCAGCCTGGAAAATA	189–231	2	3	224	1
Cln8	[AG]$_{11}$	F: ATTTCCCGGAAGTTACATGA R: CACCACCGTGTAAGGGTTTT	175–233	5	6	180	1
Cln9	[TC]$_{14}$T$_9$	F: TTGGAAGTGAAAGGAATCCAA R: TCATAAATGAGGGGCAGGAC	137–247	3	5	–	–
Total number of alleles				39	37		12

the least number of loci when compared with the 17 *Clusia* species tested by Hale et al. (2002), which might be an indication that our study species bears some phylogenetic distance from the 17 others. However, the data in Table 5.3 suggests that *C. hilariana* is indeed closer related to *C. nemorosa* than to *C. minor*, as shown by the phylogenetic trees of Vaasen et al. (2002) and Gustafsson et al. (2002) (Chap. 6) based on ITS sequence analysis. More importantly, from a population ecology viewpoint, we counted the number of alleles per loci amplified by the heterologous primers, and found a reduced polymorphism. Only *cln2, cln3*, and *clm3* were polymorphic displaying at maximum three alleles (*cln2*). These results suggest that *C. hilariana* might have a low genetic diversity in our site, at least when compared to *C. minor* and *C. ducu* studied by Hale et al. (2002). This is in agreement with the results described in Chap. 7, which also investigated genetic variation at the population level for *C. hilariana* with molecular markers. However, since the primers used in both studies were not primarily developed for *C. hilariana* specifically, the low diversity found could result from amplification of low diverse SSR regions of the DNA of our study species. Therefore, in order to reach a final conclusion about the genetic diversity of *C. hilariana* at our study site, we are currently performing new analysis using AFLP dominant markers that are independent of previous sequence knowledge and produce a larger number of information able to differentiate individuals even within a population (Kremer et al. 2005). To test this pattern further, we will compare the genetic diversity of the whole population of plants sampled for our nine sampling sites (n=90) with that of the local population of one sampling site (n=45) where the microsatellite analysis indicated the highest genetic diversity.

5.3.5 *Clusia hilariana*: A Synthesis of Ongoing Studies

In short, it appears that the high abundance of *C. hilariana* might be achieved by a combination of successful sexual reproduction in odd years with effective asexual reproduction. Eventual success in sexual reproduction is most likely related to automimicry properties, which, in the case of this plant highlights the importance of pollen as reward instead of resin. Once fruits are set and seeds dispersed, tank bromeliads will nurse seedlings and are apparently responsible for much of the success of regeneration and recruitment of seed-originated young plants. Although further confirmation is needed, there are indications that genetic diversity might be rather low, further confirming a possible important role for this type of reproduction in sustaining the high local abundance of *C. hilariana* at the RJNP.

This broad picture, however, hides processes at smaller spatial and temporal scale that account for a very large spatio-temporal variation in reproductive success of this species. For instance, spatial distribution of *C. hilariana*, particularly the distance from males to females, is inversely related to fruit set,

i.e. the shorter the distance between plants of different genders, the higher the fruit set. At the community level, distinct overall vegetation cover is related to local fruit set, but whether these variables correlate positively or negatively depends on the sampling year.

5.4 Final Remarks

The understanding about the reproductive biology of the genus *Clusia* is still in its infancy, when we consider that less than 10 % of the species were studied in this respect, and the scales of space and time covered by such studies were always reduced. However, it is quite clear at this point that variation of possible behaviours is very high, and comprises different combinations of features per species, all of which are relatively rare in nature, such as dioecy, resin production, floral mimicry, and to a lesser extent agamospermy. This high diversity and peculiarity of reproductive strategies are matched by the high diversity on floral morphology, discussed by Gustafsson et al. in Chap. 6.5. It contrasts with uniformity of leaf morphology ("one morphotype") discussed by Lüttge in Chap. 2.1.

The case of *C. hilariana* calls for the relevance of enhancing spatio-temporal sampling scales of reproductive biology studies, and this is probably true not only to the *Clusia* genus. Moreover, it also shows how reproductive biology may provide essential information to deepen our understanding of processes operating at community and ecosystem levels and vice versa. Ecology definitely benefits from integration of hierarchies and scales (Pickett et al. 1994). This plant is an exceptional curiosity: dioecious, floral mimetic, resin producer, CAM and originally a possible migrant from rain forest canopies, later to become the dominant terrestrial plant in the restinga landscape of northern Rio de Janeiro (see Chap. 3), apparently by combining regular asexual reproduction with successful sexual reproduction only in odd years, assisted by tank-bromeliads which nurse their seeds and seedlings. That such an exception might sustain biodiversity and ecosystem processes in these restingas is a marvel of nature, fortunately well-preserved within the boundaries of the Restinga de Jurubatiba National Park.

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