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ORIGINAL ARTICLE

Pollinator visitation and female reproductive success in two floral color morphs of *Ipomoea aquatica* (Convolvulaceae)

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Abstract

Differences in floral morphology are often hypothesized to stem from selection by different pollinators. Thus, the presence of multiple floral color morphs within a species might indicate visitation by different pollinator species. To test this prediction, we examined *Ipomoea aquatica* Forssk., a morning glory species with a "white" morph (all-white flowers) and a "purple" morph (white corolla lobes with purple corolla tubes). We studied this species in Nong Khai, Thailand, where some populations are monomorphic for a single color and others are polymorphic. We compared (a) animal visitation rates to each morph; (b) visitor and pollinator community composition at each morph; and (c) the female reproduction of each morph. Visitation rates were obtained from camcorder footage and used to analyze community composition. Female reproduction was assessed from a pollination experiment with five treatments (open, open emasculation, hand-cross, hand-self, and closed). We found that the main pollinators (bees and butterflies) visited both morphs, and that overall insect community composition to the two floral colors did not differ significantly. Moreover, we found that *I. aquatica* is capable of spontaneous autogamy but still benefits from pollinators, as flowers in the closed treatment set significantly fewer seeds than flowers in the open and hand cross-pollinated treatments. When comparing female reproduction between morphs, we did not find significant differences for either fruit set or seed set. These findings suggest that floral visitors interact with the two morphs similarly, and that the high-reproductive success experienced by both colors may help maintain the polymorphism in nature.

Keywords Bees · Insects · Pollination success · Polymorphism · Thailand

Introduction

The enormous diversity of floral forms observed in nature is hypothesized to stem from strong selection imposed by pollinators (Grant 1949; Stebbins 1970; Eriksson and Bremer 1992; but see Ellis and Johnson 2009). Such strong selection is unsurprising given the direct impact that pollinators have on plant reproductive success (Klein et al. 2006; Ollerton et al. 2011). In some instances, we even find diverse floral forms within a single plant species, such as when two or more floral color morphs occur (Rausher 2008). Polymorphism refers to the presence of two or more morphs within the same population, but many plant species contain both polymorphic and monomorphic populations (Narbona et al. 2018). It has been estimated that 20–25% of plant species exhibit floral color polymorphisms in England (Warren and Mackenzie 2001) and South Africa (Carlson and Holsinger 2010), with up to 40% predicted in some genera (Carlson and Holsinger 2010). Given that floral color can be an important determinant in pollinator foraging (Eckhart et al. 2006; Heystek et al. 2014), such polymorphic species raise the questions (1), how do pollinators respond to color polymorphism and (2) how does color polymorphism impact gene flow and plant reproductive success?

Previous studies have found diverse results. In some systems, pollinators exhibit distinct preferences for specific colors, as has been observed in solitary bees (Ortiz et al. 2015), hawkmoths (Streisfeld and Kohn 2007), sunbirds (Heystek et al. 2014), and hummingbirds (Elam and Linhart 1988). Other studies have found that pollinators visit all colors

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morphs indiscriminately, as has been observed in honey bees (Wolfe 1993) and bee flies (Ellis and Johnson 2009). Yet many studies have actually reported mixed results, such as observing a clear color preference in the field but a lack of assortative foraging behavior when color morphs were evenly distributed in artificial floral arrays (Stanton et al. 1989), observing a color preference at artificial arrays but not in the field (Streisfeld and Kohn 2007), finding that some species exhibit a color preference while others do not (Malerba and Nattero 2012), revealing that color preference is frequency dependent (Epperson and Clegg 1987), or reporting high-color constancy within a foraging bout but no overall color preference (Brown and Clegg 1984; Niovi Jones and Reithel 2001). Moreover, studies measuring plant reproductive success have generally reported no fitness differences between color morphs, even when pollinators exhibit significant color preferences (Elam and Linhart 1988; Wolfe 1993; Frey et al. 2011; Heystek et al. 2014; Ortiz et al. 2015; but see Schemske and Bierzychudek 2007). Further complicating matters is the fact that color polymorphisms can also be influenced by other factors such as herbivory (Irwin et al. 2003) and soil conditions (Vaidya et al. 2018). Thus, the effects of color polymorphism on pollinator foraging and plant reproductive success are still unclear.

Ipomoea aquatica Forssk. (Convolvulaceae) is commonly found throughout Southeast Asia and has two floral morphs in terms of corolla tube color, white and purple. Populations in northeastern Thailand provide a unique opportunity for studying pollinator response to floral color polymorphism, as some sites have only the white morph, some only the purple morph, and at some sites the two morphs grow intermixed. By examining the polymorphic population, we could assess whether pollinators exhibit a preference for one morph over the other. Examining monomorphic populations is also informative, as it can reveal how pollinators respond when only a single color morph is available. We therefore conducted floral observations to examine whether visitors and pollinators exhibit a color preference, or visit both morphs indiscriminately. We further compared overall community composition of both visitors and pollinators at each morph. Finally, we conducted a pollination experiment and measured fruit set and seed set to compare female reproductive output between the white and purple morphs, and to assess their dependence on animal-mediated pollen transfer. We hypothesized that some animal taxa would exhibit preferences for a specific floral morph, which would possibly impact the female reproductive success of each floral morph.

Materials and methods

Study species

Ipomoea aquatica Forssk. (Convolvulaceae), or water spinach, is a morning glory species that is native to Asia (Austin 2007) and widespread throughout Thailand (Staples and Traiperm 2010). It is a low-growing, trailing vine (Grubben and Denton 2004) commonly found in freshwater marshes and ponds (Grubben and Denton 2004; Ogunwenmo and Oyelana 2009; Staples and Traiperm 2010; Hassa pers. obs.), rice paddies, canals, and other wet or damp habitats (Staples and Traiperm 2010; Hassa pers. obs.). The leaves and stalks are commonly consumed as vegetables (Grubben and Denton 2004; Prasad et al. 2005; Meira et al. 2012), used in traditional medicine (Austin 2007; Ogunwenmo and Oyelana 2009), and supplied as fodder for cattle and pigs (Grubben and Denton 2004; Ogunwenmo and Oyelana 2009). *Ipomoea aquatica* typically produces inflorescences of 1–5 flowers, which are funnelform and have two globose stigmas with five stamens of varying lengths (Ogunwenmo and Oyelana 2009; Staples and Traiperm 2010). In our study area, flowering occurred from October through January for both floral morphs (i.e., complete phenological overlap between the two morphs) and flowers were generally open from 0530 to 1900 h (Hassa, pers. obs.). Each capsule fruit contains between 2 and 4 seeds (Grubben and Denton 2004).

Both floral morphs of *I. aquatica* are found in our study area (Fig. 1). In the morph more commonly found throughout Thailand (hereafter, the “purple” morph), the inside of the corolla gradually transitions from a deep purple at the base, to pure white at the mouth of the funnel (Fig. 1a). When viewing the external surface of the flower, the purple in the corolla shows through as a light lilac. Moreover, the stems of this morph are tinged with a reddish color (Fig. 1b) (Ogunwenmo and Oyelana 2009). In the second morph (hereafter, the “white” morph), the flowers are pure white (Fig. 1c), and the stems are a bright green (Fig. 1d) (Harwood and Sytsma 2003; Ogunwenmo and Oyelana 2009). Individual plants produce flowers of only a single color morph but, in polymorphic populations, plants of each morph grow intermixed, such that white and purple flowers are found in close proximity (Fig. 1e).

Study sites

This study was conducted in Nong Khai province in northeastern Thailand (Fig. 2), where both floral morphs occur naturally. We examined one population containing only white flowers, one population containing only purple

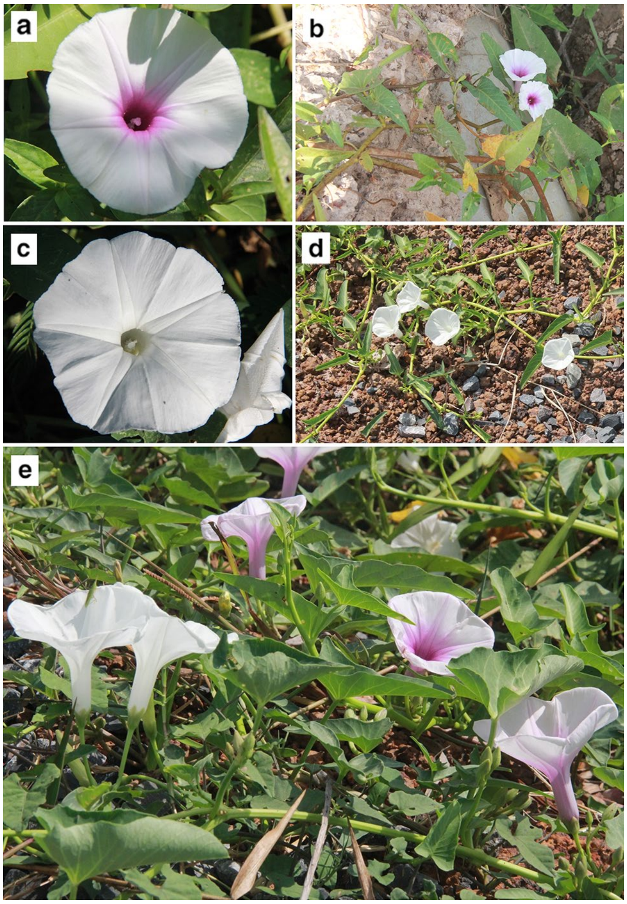

Fig. 1 Photos of *Ipomoea aquatica* morphs: **a** flower of purple morph, **b** reddish-tinged stems of purple morph, **c** flower of white morph, **d** bright green stems of white morph, and **e** both color morphs growing adjacent to each other in a polymorphic population

flowers, and one population containing both floral morphs. The three populations were all at least 60 km apart from each other.

The white population was found growing in a suburban neighborhood at a site that was damp most of the year (site "W"; 17.774219°N, 102.770930°E). At the time of data collection, there was an estimated 10 *I. aquatica* plants with approximately 50 flowers total covering an area of around 25 m^2. Site W had the highest overall plant community diversity of the three sites. Species found within a 15-m radius of site W included jackfruit (*Artocarpus heterophyllus* Lam.), Chinese violet (*Asystasia gangetica* (L.) T.Anderson), carpet grass (*Axonopus compressus* (Sw.) P.Beauv.), papaya (*Carica papaya* L.), coconut (*Cocos nucifera* L.), pumpkin (*Cucurbita* sp.), fig (*Ficus* sp.), gac (*Momordica cochinchinensis* (Lour.) Spreng.), skunk vine (*Paederia foetida* L.), pandan (*Pandanus amaryllifolius* Roxb.), red frangipani (*Plumeria rubra* L.), guava (*Psidium guajava* L.), and tomato (*Solanum lycopersicum* L.).

The purple population grew along the border of a small, shallow pond (site "P"; 18.094310°N, 102.145165°E). At the time of data collection, there was an estimated 20 *I. aquatica* plants with approximately 30 flowers total covering an area of around 400 m^2. Site P had the lowest overall plant community diversity, with only four species found within a 15-m radius: chilli (*Capsicum* sp.), papaya (*Carica papaya* L.), purple nut sedge (*Cyperus rotundus* L.), and rough horsetail (*Equisetum hyemale* L.).

Finally, the polymorphic population was found in an abandoned rice paddy (site "WP"; 18.0062222°N, 103.305083°E). At the time of data collection, there was an estimated 15 plants of the white morph (~60 flowers) and an estimated 25 plants of the purple morph (~40 flowers) covering an area of around 400 m^2. Thus, the ratio of white to purple flowers at site WP was approximately 60:40. Plant species found within a 15-m radius included finger-grass (*Digitaria* sp.), sweet potato (*Ipomoea batatas* (L.) Lam.), Aiea morning glory (*Ipomoea triloba* L.), mango (*Mangifera indica* L.), ivy woodrose (*Merremia hederacea* (Burm. f.) Hallier f.), trailing daisy (*Sphagneticola trilobata* (L.) Pruski), tamarind (*Tamarindus indica* L.) and Ceasarweed (*Urena* sp.).

The average yearly precipitation in Nong Khai is 1800 mm per year (Thai Meteorological Department, www.tmd.go.th). During our study months (October 2018–December 2018), the average monthly precipitation ranged between 0 and 75.6 mm per month (www.tmd.go.th). The mean temperature in northeastern Thailand spans from a monthly low of 18.7 °C (in December) to a monthly high of 35.2 °C (in April) (www.tmd.go.th). The average monthly temperature during our study months ranged from 24.8 to 28.2 °C (www.tmd.go.th).

Floral visitor observation

We observed floral visitors at site W over 3 days (70.4 h total) in October 2018 ($n_{\text{white}} = 19$ flowers from 5 plants) and at site WP over 3 days (60.0 h total) in November 2018 ($n_{\text{white}} = 21$ flowers from 6 plants, $n_{\text{purple}} = 18$ flowers from 6 plants). Floral visitors could not be observed at site P due to limited cameras and manpower. Animal visitors were recorded throughout anthesis using a video camera (Sony Handycam SR12); under dim or dark conditions, this model automatically switches to night-shot mode with infrared lighting. Cameras were mounted on tripods and placed by each study plant such that 3–13 flowers were in frame. For site WP, white and purple morphs grew intermixed, such that each video recording included at least one flower of each color within frame.

When reviewing the video footage, we recorded the timing of each animal's visit. Animals were identified from video footage to the lowest taxonomic level possible using

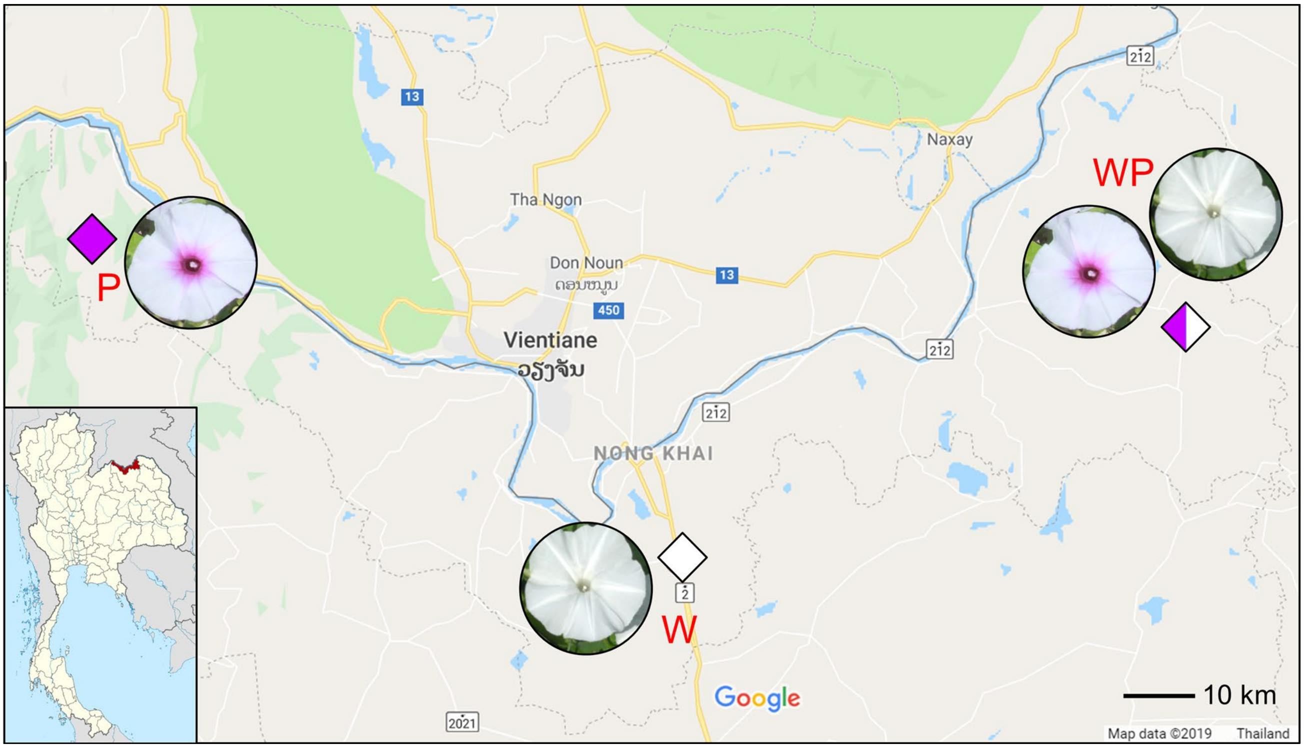

Fig. 2 The three study sites of *Ipomoea aquatica* in Nong Khai province, Thailand. Site P (purple diamond) had only purple flowers, site W (white diamond) had only white flowers, and site WP (purple and white diamond) had both floral morphs. The distance between sites P and W is 75.5 km, between sites W and WP is 62.3 km, and between sites P and WP is 123 km. In the map inset, Nong Khai province is shown in red. [Image modified from Google Maps].

Suwanphak (2013, 2016). Animals were categorized as visitor or potential pollinator based on their behavior; animals that clearly contacted floral reproductive structures were scored as pollinators, while animals that visited that flower but did not contact the stigmas and anthers were scored as visitors. Visitation rate was calculated for each animal taxa as the total number of floral visits per hour (visitor visitation rate) or the number of visits in which floral reproductive structures were contacted per hour (pollinator visitation rate).

Pollination experiment

We conducted a pollination experiment to compare the female reproductive output of each *I. aquatica* morph, and to assess their dependence on pollinators. Five treatments were used: open pollination (flowers were not manipulated and were left exposed to all visitors as normal), open emasculation (anthers were removed before anthesis and flowers were left exposed to all visitors as normal to determine how much xenogamous pollen is moved between flowers by pollinators), hand-cross pollination (virgin flowers were pollinated by hand using xenogamous pollen, and covered with fine mesh bags to prevent visitation by other pollinators), hand-self pollination (virgin flowers were pollinated by hand using autogamous pollen, and covered with fine mesh bags), and closed pollination (flowers were covered with fine mesh bags during the entire anthesis period). We selected study plants that had at least five flowers so that all five treatments could be applied to each study plant. We used five study plants of the white morph at site W in October 2018, ten study plants of the white morph and five study plants of the purple morph at site WP in November 2018, and ten study plants of the purple morph at site P in December 2018. Some flowers were lost during the course of the experiment, but we still ended up with 13–15 white flowers per treatment and 12–15 purple flowers per treatment. In total, we were able to obtain data from 139 flowers (71 white flowers and 68 purple flowers). We collected fruits 3 weeks after pollination and counted both fruit set (fruit presence or absence) and seed set (number of seeds per fruit).

Statistical analysis

All analyses were conducted in R 3.6.0 (R Core Team, 2019). To compare whether overall visitation rates (i.e., pooling data from all animal taxa) differed between the two color morphs, we performed linear mixed modeling (LMM) using the "lme4" package. We conduced separate analyses for the total visitation rate of all visitors and the total visitation rate of all pollinators using floral color and site as the fixed factors and plant ID as the random factor. We also examined whether each animal taxa exhibited significant preferences for either color using zero-inflated regression modeling (zeroinfl function in the package "pscl"), given the abundant zeros in our data (i.e., no floral visits by many taxa). Models included the number of floral visits as the response, floral color as the predictor, and total observation time as the offset variable. We used a negative binomial distribution for data that were overdispersed and a Poisson distribution for those that were not.

To compare overall community composition we conducted PERMANOVA (permutational multi-variate analysis of variance) analyses using Bray–Curtis dissimilarity with 999 permutations (adonis2 function in the package "vegan"). We conducted separate analyses for total visitor and total pollinator communities, and examined the effect of both floral color and site. We also visualized the communities using NMDS (non-metric multidimensional scaling) with a Bray–Curtis dissimilarity matrix calculated from the proportional abundance of taxa (metaMDS function in the package "vegan").

To analyze the results of the pollination experiment, we conducted generalized linear mixed modeling (GLMM; package "lme4") using floral color and treatment as the fixed factors and plant ID as the random factor. We used a binomial distribution for fruit set (fruit presence or absence) and a Poisson distribution for seed set (number of seeds per fruit). Models were compared with likelihood ratio tests, and Tukey's tests were performed for post hoc analyses (package "emmeans").

Results

Across 130.4 camera hours, we observed 1603 visits to white flower and 191 visits to purple flowers. The much greater number of visits to white flowers was driven by ants (Formicidae) and flies (Diptera); excluding these two taxa, we observed 248 visits to white flowers and 173 visits to purple flowers. A total of 36 animal taxa were observed visiting white *I. aquatica* flowers and 18 taxa were observed visiting purple *I. aquatica* flowers (Online Resource 1). Results of LMM revealed that overall visitor visitation rates were marginally influenced by site ($\chi^2_1 = 3.53$, $P = 0.060$), and not at all by floral color ($\chi^2_1 = 0.029$, $P = 0.865$). Zero-inflated regression further revealed that most of the taxa (8 out of 12 taxa that could be tested) did not exhibit a significant preference for either floral color, although 4 taxa (Formicidae sp.4, Lygaeidae spp., *Paratrechina* spp., and Dipteran unknown sp.6) significantly preferred white flowers (Online Resource 1). The most common visitors (pooling visits to both floral morphs) were Formicidae sp.4 (3.87 ± 2.01 visits/h), Dipteran unknown sp.6 (1.26 ± 1.18 visits/h), *Lasioglossum* sweat bees (1.20 ± 0.46 visits/h), Meliponini stingless bees (1.05 ± 0.37 visits/h), *Paratrechina* ants (1.00 ± 0.76 visits/h), Drosophilidae flies (0.48 ± 0.26 visits/h), *Apis* honey bees (0.18 ± 0.08 visits/h), and *Pelopidas* butterflies (0.09 ± 0.05 visits/h).

Table 1 Visitation rates (mean ± *SE*) of all pollinator taxa (animals that contacted floral reproductive structures) visiting *Ipomoea aquatica* flowers in Nong Khai, Thailand

Order	Taxa	Both colors (visits/h)		Purple flowers (visits/h)		White flowers (visits/h)		Zero-inflated regression	
		Mean	*SE*	Mean	*SE*	Mean	*SE*	*P* value	AIC
Coleoptera	Unknown sp.1	0.011	0.011	0.034	0.034	0	0	–	–
Hymenoptera	*Amegilla* sp.	0.011	0.011	0	0	0.017	0.017	–	–
	Apis spp.	0.157	0.076	0.073	0.073	0.199	0.109	0.365	53.4 (P)
	Formicidae sp.1	0.016	0.011	0	0	0.024	0.017	–	–
	Lasioglossum spp.	1.116	0.450	0.890	0.319	1.229	0.668	0.490	109.0 (NB)
	Meliponini spp.	1.002	0.379	1.361	0.576	0.822	0.501	0.864	92.8 (NB)
Lepidoptera	*Catopsilia* sp.	0.004	0.004	0	0	0.006	0.006	–	–
	Hypolimnas sp.	0.004	0.004	0	0	0.006	0.006	–	–
	Junonia sp.	0.010	0.010	0	0	0.015	0.015	–	–
	Macroglossum stellatarum	0.011	0.011	0.032	0.032	0	0	–	–
	Pelopidas spp.	0.086	0.055	0.228	0.153	0.015	0.015	0.297	31.4 (P)
Orthoptera	Gryllidae spp.	0.006	0.006	0.017	0.017	0	0	–	–

Visitation rates to both floral color morphs, purple flowers only, and white flowers only are listed, as well as the results from zero-inflated regression models determining whether or not floral color significantly influenced visitation rates. Some taxa lacked sufficient data to perform analyses (indicated by dashes). AIC values are reported for the best model (*P* Poisson distribution; *NB* negative binomial distribution)

Among the observed visitors, 9 taxa were potential pollinators of the white morph and 7 taxa were potential pollinators of the purple morph (Table 1). Considering only visits by potential pollinators, we observed 177 visits to white flowers and 149 visits to purple flowers. Results from LMM revealed that overall pollinator visitation rates were significantly influenced by site ($\chi^2_1 = 4.16$, $P = 0.041$) but not by floral color ($\chi^2_1 = 0.222$, $P = 0.638$). Moreover, zero-inflated regression revealed that none of the pollinator taxa exhibited a significant preference for either floral color (Table 1). The most common pollinator taxa (pooling visits to both floral morphs) were *Lasioglossum* sweat bees (1.12 ± 0.45 visits/h), Meliponini stingless bees (1.00 ± 0.38 visits/h), *Apis* honey bees (0.16 ± 0.08 visits/h), and *Pelopidas* butterflies (0.09 ± 0.06 visits/h).

When examining visitor community composition, the PERMANOVA results revealed that site ($F_{1,14} = 8.94$, $P = 0.002$), but not floral color ($F_{1,14} = 1.62$, $P = 0.167$), had a significant effect. Similarly, for pollinator community composition, site was significant ($F_{1,13} = 4.60$, $P = 0.003$), but not floral color ($F_{1,13} = 2.15$, $P = 0.096$). These results were mirrored by the NMDS analyses (Fig. 3), which showed high overlap between insect communities within a site, regardless of floral color (i.e., insects at white flowers and insects at purple flowers within site WP), but low overlap between insect communities visiting the same floral color at different sites (i.e., insects at white flowers within site W and insects at white flowers within site WP).

Neither treatment ($\chi^2_4 = 8.60$, $P = 0.072$) nor floral color ($\chi^2_1 = 2.49$, $P = 0.115$) significantly influenced *I. aquatica* fruit set (Fig. 4a, b). Floral color also did not have a significant effect on seed set ($\chi^2_1 = 0.62$, $P = 0.430$), but seed set did vary significantly across treatments ($\chi^2_4 = 13.5$, $P = 0.009$) (Fig. 4c, d). Post-hoc tests revealed that the closed treatment set fewer seeds than the open and hand-cross treatments ($P < 0.05$) (Fig. 4c).

Discussion

Diverse insect taxa visited *I. aquatica* flowers. The most common visitors that regularly contacted floral anthers and stigmas were three taxa of Hymenoptera (*Apis* honey bees, Meliponini stingless bees, *Lasioglossum* sweat bees) and one of Lepidoptera (*Pelopidas* butterflies). Our results are thus consistent with previous studies of Convolvulaceae, which generally report that members of this family are pollinated by bees (Bullock et al. 1987; Patiño et al. 2002; Galetto and Bernardello 2004; Maimoni-Rodella and Yanagizawa 2007; Raimúndez-Urrutia et al. 2008; Ketjarun et al. 2016; Araujo et al. 2018; Chitchak et al. 2018) or by bees and

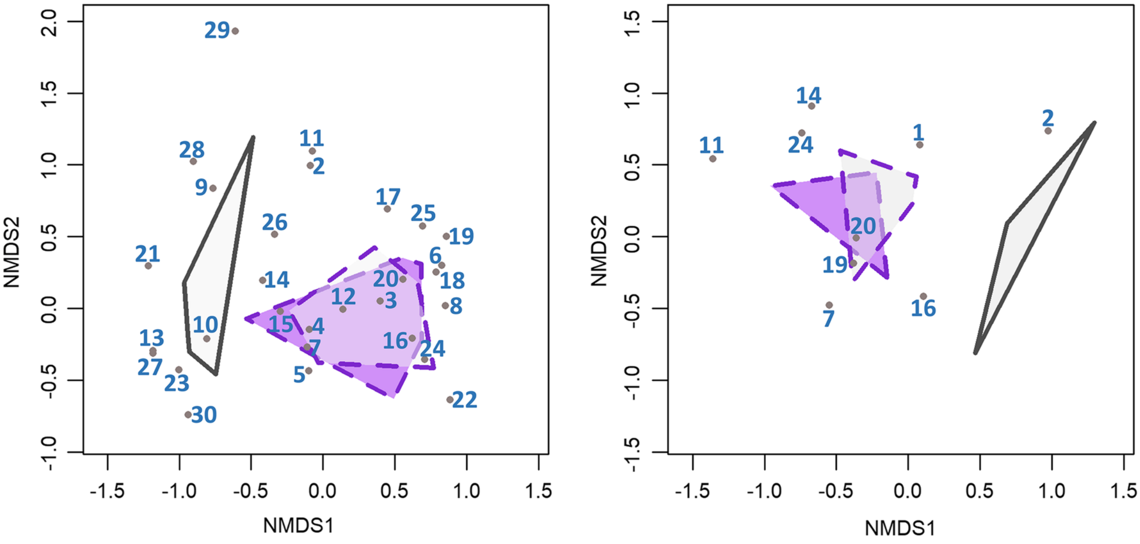

Fig. 3 Nonmetric multidimensional scaling (NMDS) showing **a** floral visitor communities and **b** pollinator communities that visited *Ipomoea aquatica* flowers. Polygons denote separate study sites; the purple dashed borders are communities at site WP (both white and purple floral morphs present), the gray solid borders are communities at site W (only white floral morphs present). Polygon fill color distinguishes between arthropod communities at purple flowers (purple fill color) and white flowers (white fill color). Both floral visitor and pollinator communities were significantly influenced by site but not floral color. Arthropod taxa: (1) *Amegilla* sp., (2) *Apis* spp., (3) Unknown sp.1, (4) Unknown sp.5, (5) Drosophilidae spp., (6) *Eristalinus arvorum*, (7) Formicidae sp.1, (8) Formicidae sp.2, (9) Formicidae sp.3, (10) Formicidae sp.4, (11) Gryllidae spp., (12) Hesperiidae sp., (13) *Hypolimnas* sp., (14) *Junonia* sp., (15) *Lampides* sp., (16) *Lasioglossum* spp., (17) Unknown sp.9, (18) Lygaeidae spp., (19) *Macroglossum stellatarum*, (20) Meliponini spp., (21) *Notocrypta* sp., (22) Unknown sp.11, (23) *Paratrechina* spp., (24) *Pelopidas* spp., (25) *Peucetia viridans*, (26) Unknown sp.3, (27) Unknown sp.10, (28) Unknown sp.6, (29) Unknown sp.12, (30) Unknown sp.13

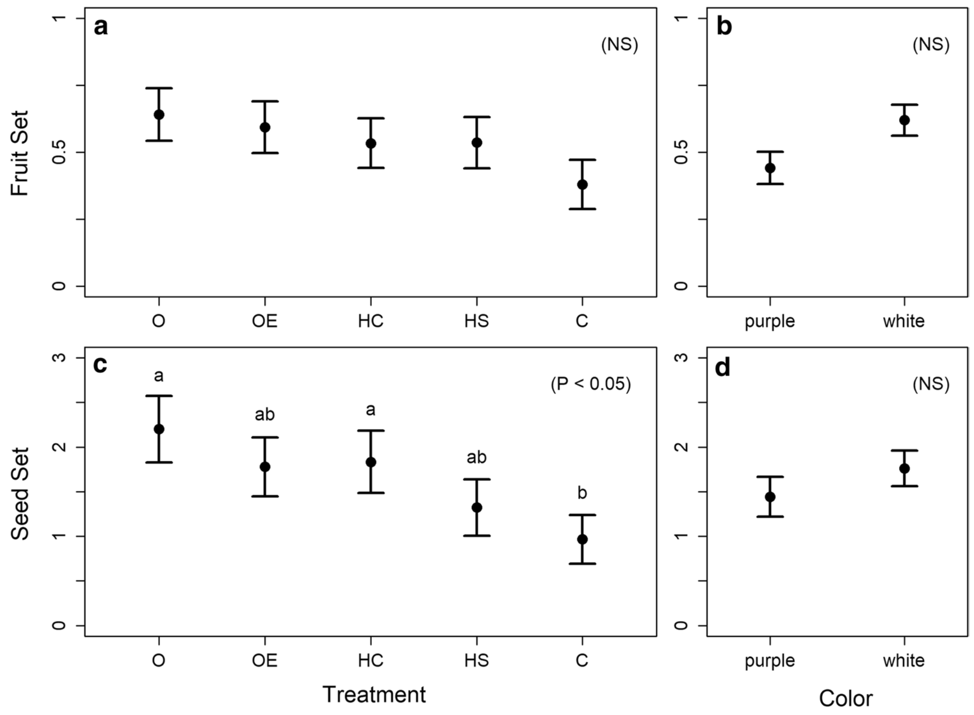

Fig. 4 Mean ± *SE* (**a**, **b**) fruit set and (**c**, **d**) seed set of *Ipomoea aquatica* across (**a**, **c**) five pollination treatments (*O* open pollination, $n = 25$ flowers; *OE* open emasculation, $n = 27$ flowers; *HC* handcross pollination, $n = 30$ flowers; *HS* hand-self pollination, $n = 28$ flowers; *C* close pollination, $n = 29$ flowers) and (**b**, **d**) two floral color morphs ($n_{\text{purple}} = 68$ flowers; $n_{\text{white}} = 71$ flowers). Treatments with different lowercase letters are significantly different. *NS* not significant ($P > 0.05$)

butterflies (Wolfe and Sowell 2006; de Souza Pacheco Filho et al. 2011).

Traits shared by *I. aquatica* flowers and those of the other aforementioned bee- and butterfly pollinated Convolvulaceae species include a funnelform shape with the floral reproductive structures retained within the narrow corolla tube. Moreover, the presence of a darker-colored corolla tube with lighter-colored corolla lobes appears to be a common color pattern among bee-pollinated *Ipomoea* species (Rosas-Guerrero et al. 2014; de Santiago-Hernández et al. 2019), including *I. bahiensis* (Araujo et al. 2018), *I. cairica* and *I. grandifolia* (Maimoni-Rodella and Yanagizawa 2007), *I. hieronymi* (Galetto and Bernardello 2004), *I. pes-caprae* (Patiño et al. 2002), and *I. wolcottiana* (Bullock et al. 1987). Such contrasting colors appear to provide a nectar guide, which has been shown to benefit bees (and the plants they pollinate) by decreasing flower handling times (Leonard et al. 2011). Among Convolvulaceae, bees appear to be less likely to visit solid white flowers, which are usually pollinated by moths (Rosas-Guerrero et al. 2014; de Santiago-Hernández et al. 2019), but bees have also been observed visiting the all-white *Merremia macrocalyx* (Raimúndez-Urrutia et al. 2008) and *Argyreia gyrobracteata* (Chitchak et al. 2018).

Interestingly, the floral visitors in our study for the most part did not appear to exhibit a preference between the white and purple morphs. Previous studies examining whether pollinators demonstrate a preference for specific color morphs reveal a broad range of findings. Malerba and Nattero (2012) found that one species of bumblebee consistently preferred pink *Cosmos bipinnatus* flowers, while a second species of bumblebee and a honey bee species showed no overall preference among the three floral colors. Stanton et al. (1989) reported that *Pieris rapae* butterflies preferred yellow over white *Raphanus raphanistrum* flowers, yet still switched between the two colors when exposed to an artificial array where the two morphs were evenly mixed. Niovi Jones and Reithel (2001) studied bumblebees visiting *Ipomoea purpurea* flowers under natural conditions and found high color constancy within a foraging bout but low color constancy over long-distance flights. Moreover, some studies using artificial flowers to examine bee color preference have found consistent preferences (Keasar et al. 1997), while others have found innate color preference to vary by colony (Dyer et al. 2016), and others have found that bees can actually acquire preferences for more rewarding colors (Goulson and Cory 1993; Gumbert 2000).

There are several potential explanations for the similar visitation rates at the white and purple morphs observed in this study. First of all, it is important to note that, while the morphs appear quite different to the human eye, we do not know how insect eyes perceive the morphs. For example, the white morph may exhibit an ultraviolet reflectance pattern that appears similar to the saturation gradient present in the purple morph, which insect vision can perceive even though human vision cannot (Papiorek et al. 2016). Moreover, it is possible that the two color morphs of *I. aquatica* are equally visible to foraging insects given that the wide mouth of the corolla is white for both morphs. In particular, if the green contrast perceived by bees is similar for both color morphs, they would be equally visible to bees at long distances since bees depend on green contrast as a far-distance signal during foraging (Giurfa et al. 1996). Finally, even if insects perceive visual differences between the two morphs, it would not be unexpected for floral visitors to visit both morphs indiscriminately if both color morphs are equally rewarding (Goulson and Cory 1993; Sanderson et al. 2006). The two morphs appear to have similar amounts of nectar and pollen, with no odor apparent to human senses (Hassa, pers. obs.), but further work should be conducted to compare the rewards (e.g., nectar and pollen) and attractants (e.g., spectral reflectance and VOCs) of each morph.

Given that insects generally did not discriminate between white and purple flowers in this study, it is not surprising that visitor and pollinator community composition did not differ between the two color morphs. Instead, we found significant differences in community composition between our study sites, which is likely due to the fact that the two sites examined (WP and W) were over 60 km apart. Previous studies have found that typical foraging ranges are around 120–850 m for stingless bees (Van Nieuwstadt and Ruana Iraheta 1996; Smith et al. 2017), no more than 12 km for honey bees (Dyer and Seeley 1991), and no more than 6 km for butterflies (Cant et al. 2005; Ovaskainen et al. 2008). Thus, it is highly unlikely that insects moved between our study sites, and habitat differences (both abiotic and biotic, including differences in plant communities) between the two sites may have accounted for their different insect communities (Janzen and Schoener 1968; Herrera 1988; Devoto et al. 2009). Additionally, insect community composition may have changed between October (when site W was observed) and November (when site WP was observed), which may account for some of the differences found between floral visitors at sites W and WP.

The finding that pollinators visited both color morphs suggests that there may be gene flow between them. From video footage at site WP, we did observe movement between color morphs, although movement between colors morphs (26 observed instances) was less common than movement between flowers of the same color (63 observed instances), as was also observed among bumblebees visiting the polymorphic *I. purpurea* (Brown and Clegg 1984). In our study, movement from white to purple flowers (13 observed instances) and from purple to white flowers (13 observed instances) were equally common. We observed the most common pollinator taxa, *Lasioglossum* sweat bees, moving between white flowers 16 times, between purple flowers 15 times, from white to purple flowers 8 times, and from purple to white flowers 8 times. We observed the second most common pollinator taxa, *Meliponini* stingless bees, moving between white flowers 3 times, between purple flowers 18 times, from white to purple flowers 4 times, and from purple to white flowers 4 times. In spite of the fact that we observed movement between color morphs, we did not observe any individuals with intermediate phenotypes, although it is possible that heterozygotes retain the dominant phenotype, as is seen in *Sisyrinchium* sp. (Takahashi et al. 2015). Thus, more research is needed to determine the extent of gene flow between the two morphs of *I. aquatica*.

Our pollination experiment revealed several findings about *I. aquatica* reproduction. Firstly, we found that *I. aquatica* in our study area is self-compatible, and even capable of spontaneous autogamy; Ogunwenmo and Oyelana (2009) previously reported that one Nigerian biotype of *I. aquatica* was self-compatible while a second was not. In our study, 8 out of 15 white flowers and 3 out of 15 purple flowers in the closed treatment set fruit, revealing that *I. aquatica* can reproduce even without pollinators. Self-pollination within a flower is likely facilitated by the fact that 1–2 stamens are equal in length to the pistil, promoting contact between anthers and stigma (the remaining stamens are shorter such that their anthers do not reach the stigma; Hassa, pers. obs.). Moreover, *I. aquatica* does not appear to exhibit dichogamy, given that flowers in the hand-self pollination treatment were pollinated as soon as flowers opened naturally, and several of these flowers set fruit. Spontaneous autogamy has been suggested as a mechanism that can maintain floral color polymorphisms (Narbona et al. 2018), and may help explain the maintenance of the two *I. aquatica* morphs. However, *I. aquatica* in our study area still benefit from pollinators, as we found that flowers in the closed treatment set significantly fewer seeds than flowers in the open and hand-cross pollinated treatments. Secondly, we found that the floral visitors are effective pollinators and that *I. aquatica* is not pollen limited, as the open and open emasculation treatments were not significantly different from the hand-pollinated treatments. Thirdly, we found that female reproductive output did not differ between the two color morphs, consistent with previous studies (Elam and Linhart 1988; Wolfe 1993; Frey et al. 2011, Heystek et al. 2014; Ortiz et al. 2015). The lack of differences in female reproduction may be another factor promoting the persistence of both floral morphs, even within the same population (Frey et al. 2011; Heystek et al. 2014).

Conclusions

Findings from numerous studies reveal that the influence of floral color polymorphism on pollinator foraging and plant reproductive success is quite varied, depending on a variety of factors such as the plant and pollinator taxa involved, any learned preferences or behaviors acquired by pollinators, and the frequency and distribution of each floral morph. In northeastern Thailand, *I. aquatica* occurs in polymorphic populations (with both purple and white morphs) as well as monomorphic populations, and it appears that both colors are equally attractive to their main pollinators (bees and butterflies). Although different floral colors are often predicted to attract different pollinators, our results suggest that floral visitors interact with the two morphs similarly in spite of different corolla tube colors. Research to date reveals that pollinator responses to floral color are diverse, and understanding these responses is important for predicting gene flow within and among plant populations, as well as discerning how they shape floral evolution and plant speciation.

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Authors' contribution This study was conceived and designed by ABS, PH, and PT; data were collected by PH; data were analyzed by ABS and PH; figures and tables were prepared by ABS and PH; the first draft was written by ABS and PH; the manuscript was revised by ABS, PH, and PT; and the final version was approved by ABS, PH, and PT.

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Availability of data Data was uploaded to a public repository (Mendeley Data): https://dx.doi.org/10.17632/vrb7wsm69z.1.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethics approval Permission to work with animals was granted by MUSC-IACUC (Faculty of Science, Mahidol University-Institutional Animal Care and Use Committee) (license number MUSC60-037-387).

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