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How frequent is external transport of seeds and invertebrate eggs by waterbirds? A study in Doñana, SW Spain

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With 2 figures and 2 tables

Abstract: Dispersal of aquatic organisms by birds has long been assumed to be an important process, but quantitative studies of its frequency are scarce. We determined the presence of plant and invertebrate propagules adhering to the plumage or feet of 47 waterbirds of 6 species (2 ducks, 2 waders and 2 rallids) trapped during the spring migration period in two localities in Doñana, south-west Spain. The percentage of waterbirds transporting propagules was high, with large differences between sites in the proportion of individuals carrying propagules (35 % and 100 %, respectively) and the numbers and types of propagules carried. Seeds of at least 15 plant species, eggs of at least 6 invertebrate species and at least one alga were encountered, with each bird carrying up to 12 different types. Seeds tended to be attached to the plumage, and invertebrate eggs to the feet. The efficiency of protocols for removing propagules from birds varied between bird and propagule species. External transport of propagules by waterbirds seems a frequent process at least at a local scale and is likely to facilitate the rapid colonisation of new or temporary wetlands, and maintain gene flow between populations.

Key words: Dispersal, egg dispersal, habitat colonisation, seed dispersal.

Introduction

The transport of plant and invertebrate propagules by adhesion has long been considered a significant mode of dispersal in aquatic environments (Darwin 1859, Ridley 1930). Darwin (1859) provided some experimental evidence of the possibility of dispersal of pond snails by adhesion. Many years later, Segerstråle (1954), showed that the amphipod *Gammarus lacustris* can ad-

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here to the plumage of ducks for up to two hours. More recently, MAKAREWICZ et al. (2001) observed that *Cercopagis pergoi* (Cladocera) can adhere to duck plumage.

Although there are a number of anecdotical observations of propagules, plant fragments or invertebrates adhering to waterbirds (reviewed in MAGUIRE 1963), quantitative analyses of the frequency of external transport are very scarce (see review by FIGUEROLA & GREEN 2002). In the only such study to date, VIVIAN-SMITH & STILES (1994) reported seeds adhering to the feathers and feet of 28 out of 36 geese and ducks, suggesting that external transport may be an important process, at least in some areas.

Here we present information on the identity and frequency of propagules adhering to waterbirds in Doñana (south-west Spain). This is the first study to consider spatial variation in the external presence of propagules in birds using different parts of a wetland complex, and to address variation between waterbird species. This is also the first study to look for differences between propagules attached to feet, and those to plumage. Finally, this is the first study to test the efficacy of protocols for detecting attached propagules, and to test for differences between waterbird species in this efficacy.

Methods

Birds were captured at two localities, Veta la Palma (6° 14′ W, 36° 57′ N) and Cañada de los Pájaros (6° 09′ W, 37° 14′ N), approximately 20 km apart within the extensive wetlands found in Doñana, SW Spain. Veta la Palma consists of a complex of c. 40 brackish ponds managed for fish farming, with a total area of 3,200 ha. It is used by many waterbirds during the winter (monthly aerial waterbird counts during the study were: January 2000, 50,709, February 26,435 and March 16,745 individuals, Estación Biológica de Doñana, unpublished data). Submerged vegetation is dominated by wigeongrass, *Ruppia maritima*, with much smaller amounts of sago pondweed, *Potamogeton pectinatus*, and the shores and islands are covered with saltmarsh vegetation, especially *Arthrocnemum* and *Suaeda*, together with small amounts of *Phragmites* and other emergent plants. Cañada de los Pájaros consists of a small, freshwater pond (c. 5 ha) resulting from the restoration of an abandoned gravel pit. The area is managed as a recreational wetland used by several hundred wintering waterbirds attracted by artificial feed. There is no visible submerged or emergent vegetation, and the steep banks are covered with terrestrial plants (chiefly Gramineae). One baited walk-in-trap was operated in each of the two localities between 28 January and 15 March 2000 (nine capture occasions in Veta la Palma, and two capture occasions in Cañada de los Pajaros). The Veta la Palma trap was 4 m square covered with poultry mesh, with funnels to allow the entrance of birds (see WAINWRIGHT 1957 and Figure 157 in BUB 1991). Rice was used to attract the birds into the traps. The trap at Cañada de los Pajaros was of the same design but larger (apr. 10 m × 4 m side). At Veta la Palma the trap was situated in a shallow unvegetated area of marsh of 10 cm depth. The trap was opened at

18:00 h and emptied at 10:00 h the following morning. At Cañada de los Pajaros the trap was set on dry land, and was opened at 21:00 h and emptied at 10:00 h the next morning. In both cases, birds entered the trap during hours of darkness. Captured birds were removed from the traps, their feet (up to the ankle) were cleaned in a plastic cup with distilled water, and they were then held individually for less than one hour in cloth bags until further processing.

Each bird was held over a plastic tray and brushed for three minutes with a soft shoe-brush. All the particles in the plastic tray were transferred to a plastic vial and transported together with the cloth bag to the laboratory for posterior examination and identification under the dissection microscope. The cloth bags were brushed in the laboratory to recover propagules that were shed from birds while in the bag. The water used to clean the feet was filtered through a 0.04 mm sieve and the material retained in the sieve examined under a binocular microscope (×25). There was a size limit for the type of propagules detectable in our study, thus organisms such as rotifers were too small to be detected by the methods used. For some individuals only samples from the feet or feather were obtained, due to insufficient cloth bags for storing samples. To prevent contamination from previous captures, cloth bags were not reused. Identification of propagules was based on CAMPREDON et al. (1982) and ALONSO (1996).

To evaluate the performance of our brushing method for recovering propagules from the plumage, we conducted some tests using captive ducks at the Wildlife Recovery Centre in Doñana National Park. Ten seeds of different plant species or ephippia of *Daphnia magna* were embedded manually in the plumage by one person. A second observer, unaware of the distribution of the seeds, applied the above protocol, and the number of propagules recovered after three minutes was counted.

Data were analysed using general linear modelling (GENMOD procedure, SAS Institute 1997), with a binomial error distribution, due to the nature of the response variable (presence/absence of propagules, or number of propagules recovered/number used in the test, see CRAWLEY 1993). The dispersion of the model was adjusted to one by scaling the model with the square root of the ratio deviance/degrees of freedom (SAS Institute 1997). For abundance data we used a negative binomial error distribution, which is more adequate for count data. Correlation between continuous variables was tested using Spearman Rank Correlations and differences in the frequency and abundance of propagules on the feet and/or the plumage were tested with Chi-Square and Wilcoxon signed-rank tests.

Results

The tests in captivity showed that the method for recovering the seeds and ephippia from plumage was highly effective. After three minutes of brushing, we recovered between 72 and 96 % of the propagules, depending on the propagule type and duck species (see Table 1). The efficiency of the method varied between species and was less efficient in the mallard than in the teal (factor species: $\chi^2 = 7.16$, 2 d.f., $P = 0.03$, post-hoc contrast $\chi^2 = 6.92$, 1 d.f., $P = 0.009$), and for *Ruppia* seeds compared to the other propagules tested (factor

Table 1. Mean ± s.e. of number of propagules recovered from tests performed on captive ducks to assess the efficiency of our protocol for collecting propagules from waterbird plumage. 10 propagules from each propagule species were inserted in the plumage of each individual bird before starting the brushing protocol (see Methods).

	Mallard *Anas platyrhynchos*	Eurasian teal *Anas crecca*	Red-crested pochard *Netta rufina*
Daphnia magna ephippia	8.67±0.41	9.57±0.43	9.40±0.60
Potamogeton pectinatus	8.78±0.22	9.00±0.38	9.20±0.58
Ruppia maritima	7.11±0.59	9.14±0.46	7.20±0.97
Scirpus maritimus	8.11±0.61	8.71±0.57	9.40±0.40
Number of ducks tested	9	7	5

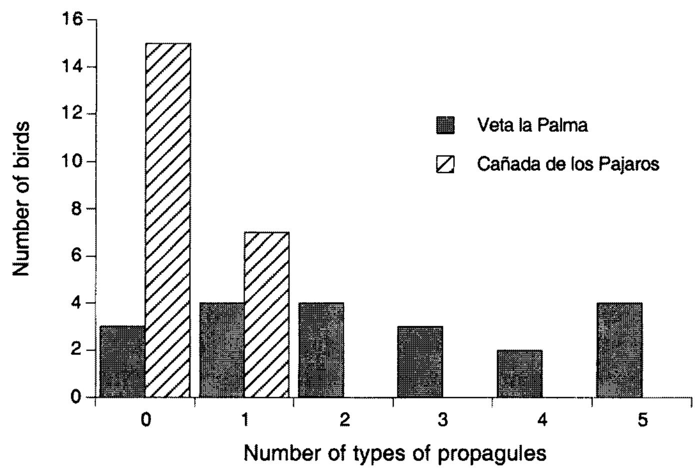

Fig. 1. Number of types of propagules found attached to the plumage of individual waterbirds at two wetlands in Doñana.

propagule, $\chi^2 = 11.48$, 3 d.f., $P = 0.009$, $P < 0.04$ for all the contrasts involving *Ruppia*). No significant interaction between propagule and duck species was detected ($\chi^2 = 7.03$, 6 d.f., $P = 0.32$).

In our field studies, propagules were more frequent in the plumage of birds from Veta la Palma (85 % of 20 individuals examined) than from Cañada de los Pájaros (45 % of 22 individuals, $\chi^2 = 7.14$, 1 d.f., $P = 0.007$). Birds captured in Veta la Palma also presented a higher number and diversity of propagules than birds captured in Cañada de los Pájaros (see Fig. 1). Restricting the analyses to species captured in both localities showed that differences in frequency and abundance of propagules varied locally (presence: $F_{1,29} = 9.18$, $P = 0.005$; abundance: $\chi^2 = 25.34$, $P < 0.0001$), but not between bird species (presence: $F_{1,29} = 0.90$, $P = 0.35$; abundance: $\chi^2 = 0.06$, $P = 0.81$). In Veta la Palma, seeds of saltmarsh species and cladoceran ephippia predominated. In the Cañada,

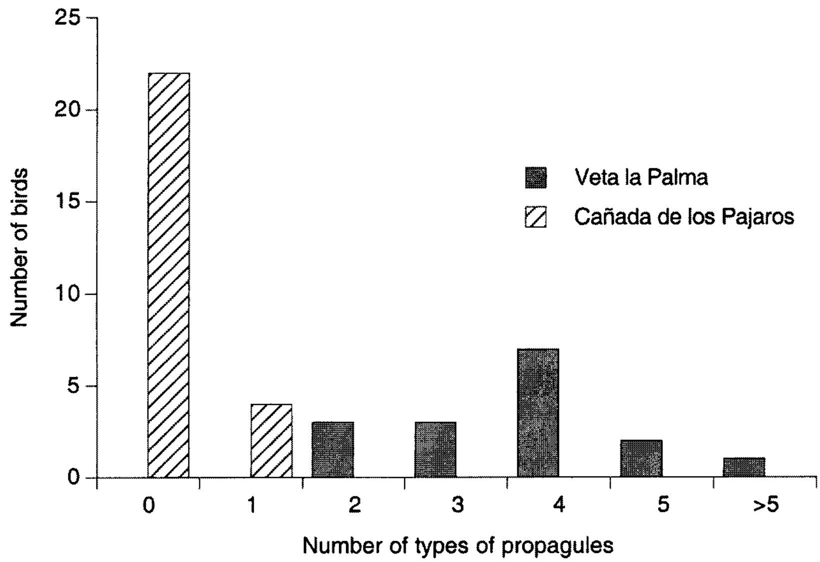

Fig. 2. Number of types of propagules found attached to the feet of individual waterbirds at two wetlands in Doñana.

seeds of terrestrial plants dominated, although with a lower overall frequency than in Veta la Palma.

From the analysis with propagules found on the feet, the same pattern emerged. Propagules were more frequent in Veta la Palma (100 % of 16 individuals examined) than in Cañada de los Pájaros (15 % of 26 individuals, χ^2 = 28.43, P <0.0001). The diversity of propagules was also higher in Veta la Palma (see Fig. 2). When analysing data for species captured in both localities, both frequency and abundance of propagules varied between sites (presence: $F_{1,34}$ = 68.47, P<0.0001; abundance: χ^2 = 42.42, P<0.0001), but not between species (presence: $F_{1,34}$ = 0.59, P = 0.45; abundance: χ^2 = 0.06, P = 0.80).

Overall, all individual birds sampled at Veta la Palma and 35 % of individuals at Cañada de los Pájaros were carrying propagules externally. The diversity of propagules in the feet and the plumage was positively correlated for Veta la Palma samples (R_S = 0.52, N = 15, P<0.05), but not in Cañada de los Pájaros (χ^2 = 0.75, 1 d.f., N = 22, P = 0.39). The characteristics of the propagules adhering to the plumage or the feet differed. A similar proportion of individuals carried invertebrate propagules attached to the feathers or the feet (χ^2 = 1.00, 1 d.f., P = 0.32). However, more individuals carried seeds in their plumage than on their feet (χ^2 = 4.00, 1 d.f., P = 0.04). Invertebrate propagules were more abundant in feet samples (Wilcoxon signed-rank paired test = 66, P<0.001), whereas seeds were more abundant in the plumage (W = 63, P = 0.02, see Table 2).

Table 2. Number of individuals examined of each waterbird species in each wetland, range of the number of propagules found on each bird, number of individual birds with propagules (in brackets) and geometric mean (in italics) of the number of propagules found on each bird carrying at least one propagule. Results are presented separately for plumage and feet samples. Geometric means were provided because this can be a best estimate of the central tendency for right skewed count data (see Sokal & Rohlf 1995). Means are not given when there was no difference in the number of propagules found on different individuals. Unid. = Unidentified, Ind. = Individuals.

		Ephippia *Daphnia magna*	Ephippia unid.	Invertebrate egg unid.	*Arthrocnemum*	*Chenopodium*	*Phalarix arundinacea*	*Ruppia*	*Salicornia*	*Scirpus littoralis*	*Scirpus maritimus*	Unid. seeds	*Chara* oospores	Algae	Ind. examined	Ind. without propagules
Veta la Palma																
Anas platyrhynchos (mallard)	Plumage	1–5 (4) *2.11*	1–6 (5) *2.93*	1–3 (3) *1.44*	1–8 (8) *2.28*	–	–	1 (2)	1–2 (2) *1.41*	–	1 (1)	1–2 (4) *1.19*	–	–	10	1
	Feet	1 (2)	1–8 (7) *3.50*	1–164 (10) *16.96*	1–4 (3) *1.59*	–	–	1 (1)	2 (1)	–	–	–	1 (2)	–	10	0
Fulica atra (coot)	Plumage	1 (1)	1–3 (4) *1.73*	3 (1)	1–3 (3) *1.44*	–	1 (1)	–	1 (2)	–	–	1–2 (2) *1.41*	–	–	5	1
	Feet	1–5 (3) *1.71*	2–20 (4) *5.18*	4–101 (6) *15.57*	1–10 (3) *2.71*	1 (2)	–	3 (1)	2 (1)	1 (1)	–	1 (1)	1 (1)	–	6	0
Recurvirostra avosetta (avocet)	Plumage	–	1 (1)	–	–	–	–	–	–	–	–	–	–	–	1	0
Tringa totanus (redshank)	Plumage	–	1–2 (3) *1.26*	1–2 (2) *1.41*	–	–	–	–	–	–	–	1 (1)	–	–	4	1
Cañada de los Pájaros																
Anas platyrhynchos (mallard)	Plumage	–	–	–	–	–	–	–	–	–	–	1 (1)	–	–	7	6
	Feet	–	–	–	–	–	–	–	–	–	–	–	–	3 (1)	10	9
Anas strepera (gadwall)	Plumage	–	–	–	–	–	–	–	–	–	–	1 (1)	–	–	2	1
	Feet	–	–	–	–	–	–	–	–	–	–	–	–	–	2	2
Fulica atra (coot)	Plumage	–	1 (2)	–	–	–	–	–	–	–	–	13 (31)	–	–	10	7
	Feet	–	–	1 (2)	–	–	–	–	–	–	–	–	–	–	11	9
Gallinula chloropus (moorhen)	Plumage	–	–	–	–	–	–	–	–	–	–	–	–	–	3	3
	Feet	–	–	–	–	–	–	–	–	–	–	1 (1)	–	–	3	2

Discussion

Like those of VIVIAN-SMITH & STILES (1994), our results indicate that adhesion of propagules to waterbirds is common in the field, and thus that external transport can be a frequent process, at least at a local scale (i.e. within a wetland complex). The potential for propagules to be transported externally may be limited by their capacity to adhere to birds, but also by their capacity to resist desiccation. Although we have not tested propagule viability, all the seeds found had external covers allowing survival outside the water. Desiccation favours hatching of *Daphnia magna* ephippia just after rehydration (DOMA 1979), and the propagules of many other invertebrates can resist desiccation (BILTON et al. 2001). Consequently, the viability of the propagules found by us in the plumage is not likely to have been strongly reduced during transport by birds.

The local differences in presence of propagules on the waterbirds were consistent between different bird species and can be explained by at least three different factors. First, the substratum in Veta la Palma consists of sticky mud, whereas in Cañada de los Pájaros it is sandy and less sticky. The mud in Veta la Palma may have facilitated the adhesion of propagules to the feathers and feet of the birds. Second, the shoreline in Veta la Palma is densely vegetated, whereas shoreline vegetation is scarcer in the Cañada, where the artificial pond is surrounded by terrestrial vegetation. This probably resulted in more contact between waterbirds and vegetation in Veta la Palma, translating into higher rates of adhesion of propagules. Third, propagules may be scarcer in the Cañada, a relatively simpler environment. This illustrates a possible drawback of extrapolating from data obtained from artificial wetlands with a low diversity, because bird-mediated dispersal may be less frequent in these systems.

The small size of many invertebrate propagules, in combination with the presence of mud, could explain their higher presence in feet samples. Many of the recovered seeds have hairs or serrations that could have facilitated their adhesion to feathers (e.g. *Salicornia, Arthrocnemum*), and explain their greater abundance in plumage than in feet samples. Interestingly, seeds apparently lacking any adaptation for dispersal by adhesion (as identified by SORENSON 1986) were also recovered, although at a much lower frequency than in the droppings of ducks in the same population (e.g. *Ruppia*, see FIGUEROLA et al. 2001).

Within the limited range of waterbird species examined by us, no interspecific differences in the frequency or abundance of propagules adhered was found, although analyses of a more diverse avian sample probably may produce contrasting results. We suggest that future studies of external transport should include tests of the efficiency of the protocols used to remove propagules. Our tests show that the efficiency varies between bird and propagule species, and such biases may potentially influence the apparent differences in abundance of different propagules attached to different bird species. However, these biases are not likely to affect the main conclusions of our study. We also suggest that future studies include tests of the effects of trapping methods on the abundance of adhered propagules. We cannot rule out the possibility that some propagules became detached or attached to birds between entering our traps and being screened. Experiments adhering propagules to birds then placing them in the trap overnight to determine propagule loss inside the trap, or examining the presence of propagules in birds emptied of propagules and kept inside the trap overnight would give relevant information to quantify propagule detachment-attachment inside the traps.

The capacity for external long-distance transport depends largely on the migratory behaviour of the waterbird concerned. Amongst the species included in this study, the waders migrate most and the moorhens the least (Cramp & Simmons 1977, 1983). In any case, the mobility of all the bird species sampled is high enough to facilitate the dispersal of many aquatic organisms between different wetland complexes or catchments. The implications of external transport for population dynamics and community diversity in aquatic communities clearly merits further research.

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