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Internal transport of seeds by migratory waders in the Odiel marshes, south-west Spain: consequences for long-distance dispersal

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Waders (Charadriiformes) undergo particularly long migratory flights, making them ideal vectors for long-distance dispersal. We present a study of dispersal of plant seeds by migratory waders in the Odiel saltworks in south-west Spain. This is the first field study to demonstrate excretion of viable seeds by waders. Viable seeds of *Mesembryanthemum nodiflorum* (Aizoaceae), *Sonchus oleraceus* (Asteraceae) and *Arthrocnemum macrostachyum* (Chenopodiaceae) were frequent in pellets and faeces of redshank *Tringa totanus*, spotted redshank *Tringa erythropus*, and black-tailed godwit *Limosa limosa* during spring and autumn migrations, but less frequent during winter. Another 11 seed types were recorded at low density. More intact *M. nodiflorum* seeds were present in redshank faeces than in their pellets, but seeds extracted from pellets were more likely to germinate. More *S. oleraceus* seeds were transported per redshank pellet in spring, but more redshank migrated through the area in autumn. The distributions of the plants transported are consistent with an important role for long-distance dispersal by waders. *M. nodiflorum* and *S. oleraceus* are introduced weeds in the Americas and Australasia, and dispersal by birds may contribute to their rapid spread. Although *S. oleraceus* is generally thought to be wind-dispersed, birds may be responsible for longer distance dispersal events.

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The Charadriiformes include species undergoing the longest migrations of any animal (del Hoyo et al. 1996). Darwin (1859) proposed that migratory waterbirds played a major role in the dispersal of plant seeds, and waders are likely to be particularly important owing to their long-distance migrations. Seeds lacking a fleshy fruit are often consumed by waders (Green et al. 2002) and studies in captivity suggest they can retain viability following gut passage (Figuerola and Green 2002). Proctor (1962) demonstrated transport of viable *Chara* oospores by waders in Texas. However, as far as we know, dispersal of viable angiosperm seeds by waders has never been demonstrated in the field.

In this study we document internal dispersal of seeds by waders migrating through the Odiel marshes in southwest Spain, quantifying numbers of intact seeds and assessing their viability. We quantify levels of dispersal by different wader species at different times of the year, comparing the potential for long-distance dispersal during spring and autumn migrations. Ours is the first field study to examine interspecific and seasonal variation in the abundance of viable seeds in wader excreta.

Methods

A total of 140 faecal samples and 272 pellets were collected from 2001 to 2003 from waders in the saltworks of the Odiel marshes in Huelva province (37° 17′N 06° 55′W, area 7,158 ha), a site of international importance for migratory waders migrating through the East Atlantic flyway (Stroud et al. 2004, Sánchez et al. in press). For the purpose of seasonal comparison, sampling dates of 18 March and 23 April 2001, and 7 April 2003 were considered as spring, from 22 July to 21 August as summer, from 5 September to 18 October as autumn and 13 January plus 28 February 2001 as winter. Spring samples coincided with the beginning of the northwards wader migration, and summer and autumn samples with the southwards migration (Sánchez et al. in press). Pellets and faeces were collected from redshank *Tringa totanus*, pellets from spotted redshank *Tringa erythropus*, and faeces from the black-tailed godwit *Limosa limosa* (a species that does not regurgitate pellets). These species were selected because of their abundance in the study area and because their droppings and pellets samples are large, helping to avoid soil contamination. Redshank and black-tailed godwit are two of the four most numerous waders at this site (Sánchez et al. in press).

We collected fresh faeces and pellets from roost sites on dykes in saltworks used by monospecific flocks at high tide. Collection points were observed for 30 minutes prior to sampling and only fresh samples were taken to ensure species identification. In spring 2003, 52 pellet samples were collected from a mixed flock of the two *Tringa* species. Each sample of excreta was carefully separated from the soil and placed in a tube. Given the number of birds present, we are confident that each sample was from a different bird.

The samples were stored at 5° C, until the time of analysis (a lapse of one week for samples collected in 2003, and of six to 12 months for the rest). Seeds were extracted by washing in a 0.04 mm sieve and flotation in hypersaline brine. They were counted, washed in distilled water then dried for 48 h at 40° C, followed by storage at 5° C prior to germination experiments. Seeds were considered intact when they retained an intact seed coat.

Not all samples were stored so as to retain their viability, so that germination was tested for a subset of samples. Extracted seeds were placed on moist filter paper in a petri dish and watered regularly with distilled water. They were placed inside a germination chamber at 22° C on a 12D:12L photoperiod, on 2 October 2003. They were checked daily for germination for 30 days. One sample of 84 seeds from a redshank pellet was attacked by mould and removed from the experiment. Some seedlings were cultivated to enable species identification.

Owing to the high proportion of zeros in the sample and severe problems of over-dispersion and model convergence, we were unable to conduct satisfactory parametric analyses of the numbers of seeds in samples in relation to differences between season, wader species or sample type. We therefore used non-parametric Kruskal-Wallis and Mann-Whitney U tests employing Statistica 5.5 (StatSoft 1999).

In 12 different salt ponds representing 27.6% of the 1174 ha of saltworks, we counted the number of shorebirds of each species one day each week throughout 2001. We carried out a count of three hours duration around high tide when the densities of waders are highest (Sánchez et al. in press).

Results

Intact angiosperm seeds were recorded in the droppings and/or pellets of all three wader species and in all seasons (Table 1). Overall, they were recorded in 21.8% of samples with 3.19 ± 0.94 intact seeds per sample (mean $\pm$ S.E. for those samples with at least one seed, $n = 90$). In total, 74.2% of all seeds recorded were intact (Table 1).

A total of 14 seed species were recorded. The slender-leaved iceplant *Mesembryanthemum nodiflorum* L. (Aizoaceae) was particularly abundant in redshank faeces in autumn. The common sowthistle *Sonchus oleraceus* L. (Asteraceae) was particularly abundant in spring pellets of redshank and spotted redshank. *Arthrocnemum macrostachyum* (Moric.) K. Koch (Chenopodiaceae) was the only species recorded in samples from all combinations of wader species and seasons (Table 1). All three plant species are abundant on the dykes of Odiel salt pans. Other intact seeds recorded were *Suaeda* spp. (one seed), *Salicornia* spp. (two seeds), *Cerastium* spp. (one seed) and eight unidentified taxa (one seed of each).

The proportion of all seeds that were intact was 41.0% for *A. macrostachyum*, 54.3% for *M. nodiflorum* and 84.3% for *S. oleraceus* (Table 1). The maximum number of intact seeds in one sample was 83 *S. oleraceus* seeds in a redshank pellet collected on 18 March 2001. There were highly significant differences between seasons in the

Table 1. Abundance of *A. macrostachyum*, *M. nodiflorum*, *S. oleraceus* and other seeds in wader droppings and pellets from the Odiel salt pans, listing the numbers of samples with seeds (including those broken) and intact seeds. The total number of seeds in those samples are given in parentheses. Each sample contains only a small fraction of the seeds excreted in a 24 h period by a given bird.

				A. macrostachyum		*M. nodiflorum*		*S. oleraceus*		Others		Total	
			n	Seeds	Intact	Seeds	Intact	Seeds	Intact	Seeds	Intact	Seeds	Intact
Tringa totanus	drop.	Aut-01	84	3 (3)	2 (2)	22 (29)	13 (16)	1 (1)	1 (1)	2 (2)	2 (2)	25 (35)	16 (21)
	pell.	Spr-01	33	5 (10)	3 (4)	1 (2)	1 (2)	12 (155)	12 (144)	0 (0)	0 (0)	16 (167)	15 (150)
		Aut-01	157	6 (7)	3 (3)	2 (2)	2 (2)	0 (0)	0 (0)	8 (7)	7 (8)	15 (16)	12 (13)
		Win-01	21	2 (1)	0 (0)	2 (2)	1 (1)	0 (0)	0 (0)	1 (1)	0 (0)	5 (4)	1 (1)
Limosa limosa	drop.	Sum-01	42	2 (2)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	3 (3)	1 (1)
	drop.	Sum-02	14	1 (1)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	1 (1)	0 (0)	2 (3)	0 (0)
Tringa erythropus	pell.	Spr-01	9	4 (6)	2 (2)	0 (0)	0 (0)	6 (10)	5 (7)	0 (0)	0 (0)	6 (16)	5 (9)
Tringa spp.	pell.	Spr-03	52	5 (8)	4 (4)	0 (0)	0 (0)	15 (31)	8 (13)	1 (1)	1 (1)	18 (40)	12 (18)
Total			412	28 (38)	15 (16)	27 (35)	17 (21)	35 (198)	26 (165)	14 (13)	10 (11)	90 (284)	62 (213)

abundance of intact *S. oleraceus* seeds in redshank pellets (Kruskal-Wallis test, $H = 68.22$, $df = 2$, $n = 211$, $P < 0.0001$). They were only recorded in spring, when they were significantly more abundant than autumn (Mann-Whitney U test, $U = 1648.5$, $P < 0.0001$) or winter ($U = 220.5$, $P = 0.0021$). A similar and marginally significant seasonal trend was recorded in the abundance of intact *A. macrostachyum* seeds ($H = 5.82$, $df = 2$, $P = 0.055$). In autumn, intact *M. nodiflorum* seeds were significantly more abundant in redshank faeces than in their pellets (Mann-Whitney U test, $U = 5654$, $P < 0.0001$). There were no significant differences in the abundances of intact seeds of the three species between spring pellets of redshank and spotted redshank (Mann-Whitney U tests, $P > 0.5$).

Overall, 45.5% of *A. macrostachyum*, 23.5% of *M. nodiflorum* and 76.3% of *S. oleraceus* seeds germinated (Table 2). Amongst autumn redshank samples, *M. nodiflorum* seeds from pellets were significantly more likely to germinate than those from faeces (Fisher exact test, $P = 0.0385$).

Discussion

This represents the most extensive study to date of the internal transport of plants by waders and suggests that waders disperse large quantities of viable seeds. We have found black-tailed godwits, redshank and spotted redshank to be effective dispersers of three plant species at the Odiel marshes, where thousands of waders pass through on migration (Wetlands International 2002, Stroud et al. 2004). The East Atlantic Flyway, used by an estimated 15.5 million shorebirds (including 440,000 redshank, 200,000 black-tailed godwit, and 50,000 spotted redshank, Stroud et al. 2004), is likely to be a major means of long-distance dispersal of plants. Ringing recoveries (from 1992 to 2003) show that redshank and godwit stopping in Huelva province share a common flyway, with six redshank recoveries from France, six from the Netherlands, one from Belgium, one from Germany and one from Sweden. There were six godwit recoveries from the Netherlands and one from Germany. Colour ringed birds from the Icelandic breeding population of black-tailed godwit have also been recorded at Odiel (T. G. Gunnarsson, J. A. Gill and P. M. Potts, unpubl. data). Seeds are also likely to be transported internally by some of the other 24 migratory wader species using this flyway (Stroud et al. 2004).

The birds we studied included a mixture of birds that had just completed or were just about to commence long-distance movements, and birds that were making movements between feeding and roosting sites within our study sites. The average time spent by shorebirds at stopover sites during migration varies from one to 50 days and is unknown for sites in south-west

Table 2. Germination of *A. macrostachyum*, *M. nodiflorum*, *S. oleraceus* and other seeds in wader droppings and pellets from the Odiel salt pans, listing numbers of samples with intact seeds placed for germination, and with at least one seed germinating. The total numbers of seeds involved are given in parentheses. Sample sizes are lower than Table 1 because not all samples were stored so as to conserve viable seeds.

			A. macrostachyum		*M. nodiflorum*		*S. oleraceus*		Others		Total	
			Intact	Germinated	Intact	Germinated	Intact	Germinated	Intact	Germinated	Intact	Germinated
Tringa totanus	drop.	Aut-01	1 (1)	1 (1)	11 (14)	1 (1)	0	0	0	0	12 (15)	2 (2)
	pell.	Spr-01	2 (2)	0	0	0	11 (60)	11 (54)	0	0	13 (62)	11 (54)
		Aut-01	2 (2)	1 (1)	2 (2)	2 (2)	0	0	3 (3)	0	7 (7)	3 (3)
		Win-01	0	0	1 (1)	1 (1)	0	0	0	0	1 (1)	1 (1)
Limosa limosa	drop.	Sum-01	0	0	0	0	0	0	0	0	0	0
	drop.	Sum-02	0	0	0	0	0	0	0	0	0	0
Tringa erythropus	pell.	Spr-01	2 (2)	1 (1)	0	0	5 (7)	3 (4)	0	0	5 (9)	3 (5)
Tringa spp.	pell.	Spr-03	4 (4)	2 (2)	0	0	8 (13)	8 (13)	1 (1)	1 (1)	12 (18)	11 (16)
Total			11 (11)	5 (5)	14 (17)	4 (4)	24 (80)	22 (71)	4 (4)	1 (1)	50 (112)	31 (81)

Iberia. One of two icelandic godwits seen at Odiel on autumn migration was still there 36 days later (T. G. Gunnarsson, J. A. Gill and P. M. Potts, unpubl. data). It is currently impossible to assess what proportion of the seeds transported in faeces or pellets by waders at Odiel would undergo long-distance dispersal. Godwits and other shorebirds regularly move between salt pans and other wetlands separated by up to 20 km while at stopover sites (Farmer and Parent 1997, P. M. Potts, pers. comm.), thus facilitating seed dispersal between different parts of a wetland complex.

Shorebirds move rapidly between coastal wetlands, and distances between stopovers can easily exceed 1,000 km (Iverson et al. 1996, Pennycuick and Battley 2003). Black-tailed godwits, redshank and other waders are thought to fly non-stop between Odiel and northern Europe (Beintema and Drost 1986). Redshank and godwits fly at 56-60 km h^{-1} (Welham 1994). Laboratory studies of gut passage of intact seeds suggests that they may be transported over great distances, with maxima of well over 1000 km. In a laboratory study of 13 seed and three wader species, maximum retention time varied from 3 h to 14 days (Proctor 1968). Species such as godwits that do not regurgitate compacted pellets from the gizzard may regurgitate seeds on their own from the gizzard after extraordinary intervals (Proctor 1968). The modal retention time (much less than the mean) of *Amaranthus* and *Sagittaria* seeds defecated by the killdeer *Charadrius vociferus* (size intermediate between redshank and godwit) was 1–2 h (deVlaming and Proctor 1968).

We found that *M. nodiflorum* seeds were more abundant in faeces than in pellets. This is likely to further long distance dispersal, as propagules are likely to be retained longer when expelled in faeces rather than in pellets (Nogales *et al.* 2001). In contrast, *M. nodiflorum* seeds extracted from pellets were more likely to germinate, suggesting that longer retention times involved in passage through the intestines reduces viability.

Ours is the first field study to demonstrate excretion of viable angiosperm seeds by waders. Only a minority of seeds seem to be digested (Table 1), and seeds appear to be of marginal importance in wader diets at Odiel (Sánchez et al. 2005). Overall, we recorded seeds (whether intact or not) in 22% of our samples. The abundance of seeds recorded in other studies of wader diet (Green et al. 2002, Montalti et al. 2003) suggests that waders play a widespread and important role in the dispersal of many plant species. Darwin (1859) proposed that dispersal of propagules by migratory birds such as waders plays a vital role in explaining the distribution of plant species. However, he underestimated the capacity of seeds lacking a fleshy fruit to survive passage through the digestive tract, and imagined that long-distance dispersal by internal transport may require a role for birds of prey in extracting seeds from a bird's crop before they enter the gizzard. Our study shows that waders can disperse seeds without the need for such rare predation events.

The native distribution of dispersed species included in this study (Tutin et al. 2001) is consistent with a role of long-distance dispersal by waders to and from sites such as the Odiel marshes. Wader species differed in their migration phenology at Odiel. Redshank had a smaller peak during spring migration in April (up to 996 birds), and a larger one between July and October (up to 2,170) during autumn migration. Spotted redshank numbers peaked in October (up to 351) with a lower peak in April (up to 159). Godwit peaked from July to August (up to 6,684) at the beginning of autumn migration. Taking into account the frequencies of viable seeds recorded in different seasons, and the numbers of birds moving through the Odiel saltworks at different times, our data suggest that there should be greater long distance dispersal of *S. oleraceus* northwards from Odiel, and greater dispersal of *M. nodiflorum* and *A. macrostachyum* in a southerly direction. *S. oleraceus* is widespread across the range of ringing recoveries as far north as Sweden. It has also been recorded in Iceland where it is considered an alien (A. Gardarsson, pers. comm.), and is native down to southern Africa which is visited by some redshank and other waders breeding in Europe (Wetlands International 2002). *M. nodiflorum* is considered a Mediterranean species but is also native in southern Africa. *A. macrostachyum* is native across the Mediterranean and down to Mauritania, a major wintering ground for waders passing through Odiel (Stroud et al. 2004).

Stopover times and the probabilities that waders move between suitable habitats for the dispersed species are likely to differ between spring and autumn migration, and to vary between wader species, but there are currently no data on these important issues. Furthermore, dunlin *Calidris alpina* and little stint *Calidris minuta* are much more abundant at Odiel during spring than autumn migration, and it is possible that these species are also good dispersers of plants.

S. oleraceus and *M. nodiflorum* are also widespread invasive exotics in the Americas, and Australasia (Villaseñor et al. 2004, http://plants.usda.gov). Dispersal by migratory waders may play a previously unrecognised role in the spread of these invasive weeds. *S. oleraceus* seeds have been recorded in bird droppings in the introduced range (McGrath and Bass 1999). However, *S. oleraceus* is generally assumed to be a wind-dispersed species (Jakobsson and Eriksson 2003). Our study supports the proposal of Higgins et al. (2003) that birds can sometimes be the main means of long-distance dispersal for seeds that appear morphologically adapted for wind dispersal.

In summary, dispersal via waders is likely to have a significant role in the biogeography of plants. More detailed studies are required before the true significance of this dispersal can be established and compared to that of other vectors such as wind, water or man. In particular, more data are required on the role of wader species not included in this study, and on the probabilities that propagules are dispersed to suitable habitats (Nathan et al. 2003).

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References

Beintema, A. J. and Drost, N. 1986. Migration of the black-tailed godwit. – Gerfaut 76: 37–62.

Darwin, C. 1859. On the origin of species by means of natural selection. – John Murray, London.

del Hoyo, J., Elliott, A. and Sargatal, J. (eds) 1996. Handbook of the Birds of the World vol. 3. – Lynx Edicions, Barcelona.

de Vlaming, V. and Proctor, V. W. 1968. Dispersal of aquatic organisms: viability of seeds recovered from the droppings of captive killdeer and mallard ducks. – Am. J. Bot. 55: 20–26.

Farmer, A. H. and Parent, A. H. 1997. Effects of the landscape on shorebird movements at spring migration stopovers. – Condor 99: 698–707.

Figuerola, J. and Green, A. J. 2002. Dispersal of aquatic organisms by waterbirds: a review of past research and priorities for future studies. – Fresh. Biol. 47: 483–494.

Green, A. J., Figuerola, J. and Sánchez, M. I. 2002. Implications of waterbird ecology for the dispersal of aquatic organisms. – Acta Oecol. 23: 177–189.

Higgins, S.I., Nathan, R. and Cain, M.L. 2003. Are long–distance dispersal events in plants usually caused by nonstandard means of dispersal? – Ecology 84: 1945–1956.

Iverson, G. C., Warnock, S. E., Butler, R. W., Bishop, M. A. and Warnock, N. 1996. Spring migration of western sandpipers along the Pacific Coast of North America: a telemetry study. – Condor 98: 10–21.

Jakobsson, A. and Eriksson, O. 2003. Trade-offs between dispersal and competitive ability: a comparative study of wind-dispersed Asteraceae forbs. – Evol. Ecol. 17: 233–246.

McGrath, R.J. and Bass, D. 1999. Seed dispersal by Emus on the New South Wales north-east coast. – Emu 99: 248–252.

Montalti, D., Arambarri, A., Soave, G. E., Darrieu, C. A. and Camperi, A. 2003. Seeds in the diet of the white-rumped sandpiper *Calidris fuscicollis* in Argentina. – Waterbirds 26: 166–168.

Nathan, R., Perry, G., Cronin, J. T., Strand, A. E. and Cain, M. L. 2003. Methods for estimating long-distance dispersal. – Oikos 103: 261–273.

Nogales, M., Medina, F.M., Quilis, V. and González-Rodríguez, M. 2001. Ecological and biogeographical implications of yellow-legged gulls (*Larus cachinnans* Pallas) as seed dispersers of *Rubia fruticosa* Ait. (Rubiaceae) in the Canary Islands. – J. Biogeogr. 28: 1137–1145.

Pennycuick, C. J. and Battley, P. F. 2003. Burning the engine: a time-marching computation of fat and protein compsuption in a 5420-km non-stop flight by great knots, *Calidris tenuirostris*. – Oikos 103: 323–332.
Proctor, V. W. 1962. – Viability of *Chara oospores* taken from migratory water birds. –Ecology 43: 528–529.
Proctor, V. W. 1968. Long-distance dispersal of seeds by retention in digestive tract of birds. – Science 160: 321–322.
Sánchez, M.I., Green, A.J. and Castellanos, E.M. 2005. Seasonal variation in the diet of the redshank *Tringa totanus* in the Odiel Marshes, south-west Spain: a comparison of faecal and pellet analysis. – Bird Study 52: 210–216.
Sánchez, M. I., Green, A. J. and Castellanos, E. M. Spatial and temporal fluctuations in use by shorebirds and in availability of chironomid prey in the Odiel saltpans, south-west Spain. – Hydrobiologia in press.
StatSoft 1999. Statistica 5.5. – StatSoft Inc., Tusla.
Stroud, D. A., Davidson, N. C., West, R., Scott, D. A., Haanstra, L., Thorup, O., Ganter, B. and Delany, S. 2004. Status of migratory wader populations in Africa and Western Eurasia in the 1990s. – Int. Wader Stud. 15: 1–259.
Tutin, T.G., Heywood, V. H., Burges, N. A., Valentine, D. H., Walters, S. M. and Webb, D. A. 2001. Flora Europaea. – Cambridge University Press.
Villaseñor, J. V. and Espinosa-García, F. J. 2004. The alien flowering plants of Mexico. – Div. Distrib. 10: 113–123.
Welham, C. V. J. 1994. Flight speeds of migrating birds: a test of maximum range speed predictions from three aerodynamic equations. – Behav. Ecol. 5: 1–8.
Wetlands International. 2002. Waterbird population estimates – third edition. –Wetlands International, Wageningen.

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