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Running head: *At-risk mowing areas for Corn Crake chicks*

Use of microsatellite-based paternity assignment to establish where Corn Crake *Crex crex* chicks are at risk from mechanised mowing

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We used microsatellite DNA to assign probable parentage of young Corn Crakes to adult males and females and use these assignments to estimate the distribution of distances between broods of chicks and juveniles and the night-time singing place of the father at the time of initiation of the clutch. Estimated distances for broods of young chicks were in accord with those estimated previously by radio-tracking, but distances were greater for older unfledged independent chicks not studied previously. Our results indicate that modifications of the

timing and method of mowing to reduce losses of nests and chicks should be implemented inside an area within about 500 m of the singing places of male Corn Crakes, rather than the 250 m previously considered to be safe.

Keywords: age-related movement change, agri-environment, conservation management, ranging behaviour.

The Corn Crake *Crex crex* is a migratory rail which breeds in tall vegetation in Eurasia. Populations in western Europe, including the UK, declined markedly, co-incident with the introduction of mechanised mowing of grass (Norris 1947, Green 1995, Green *et al.* 1997a), which destroys nests and kills chicks (Norris 1947, Tyler *et al.* 1998). The Corn Crake is red-listed in the UK Birds of Conservation Concern assessment (Eaton *et al.* 2015) because of its decline, but a partial recovery since the 1990s coincided with encouragement to farmers, through payments from conservation bodies and government agri-environment schemes, to delay mowing and to adopt Corn Crake-friendly mowing methods (O'Brien, *et al.* 2006). The latter at least halves the proportion of chicks killed by mowing (Green *et al.* 1997b, Tyler *et al.* 1998). Knowledge of the location of nesting adult female Corn Crakes and their flightless chicks would be useful for targeting these actions, but the only practical way to determine locations of Corn Crakes is to survey singing adult males at night. Radio-tracking of adult male and female Corn Crakes in Scotland showed that both sexes were often sequentially polygamous and formed short-term pair bonds during which the female laid eggs in a nest close to (range 45–160 m; mean 101 m; $N = 9$) the night-time singing place of the male (Tyler & Green 1996). Radio-tagged females with chicks ($N = 32$) used a small brood-rearing area (mean extent of 3.2 ha) around the nest site during the period of dependence (12-18 days) (Tyler 1996), but less is known of the movements of chicks between independence and fledging at about 45 days of age. Most females produced two broods of young per year and incubated their eggs and reared their young hidden in tall vegetation (Green *et al.* 1997b). Females, nests and young cannot be surveyed by any known method. The distribution of nests and young might therefore differ from that of males.

In this paper, we use paternity assignments of captured chicks and juveniles, based upon DNA sampling of the young and adult males, to estimate distances between unfledged chicks at risk from mowing and the singing place of their father. We assess the implications of these results for the conservation management of Corn Crake breeding areas.

METHODS

Surveying, catching and sampling singing adult male Corn Crakes

We studied a re-introduced Corn Crake population at the Nene Washes (52.58°N, 0.07°W) in Cambridgeshire, England, UK, centred on a nature reserve owned and managed by the Royal Society for the Protection of Birds (RSPB). Night-time surveys of singing male Corn Crakes were conducted in May–July of 2013, 2014 and 2015, commencing when Corn Crakes arrived in the breeding area from their spring migration (Table 1). As many of the males as possible were captured at night by luring them into mistnets using a broadcast recording of conspecific song. Each bird was marked individually with a numbered BTO metal ring, or a previously applied ring was read, and a sample of buccal epithelial cells obtained using a cotton swab. Appendix S1 gives further details of the study area and methods.

Drive catching and sampling of adults, chicks and juveniles

Corn Crake adults, chicks and juveniles were captured by driving them into funnel traps in July-August. For each drive, an approximately rectangular area of 1.2 – 4.7 ha of tall grass and herbage was enclosed by a combination of fences of netting and existing barriers, such as water-filled ditches. Corn Crakes within it were driven towards a line of traps linked by drift

fences set at one end of the drive area. It was not possible to conduct drive catches over the whole study area, but drive areas were widely spread. Further details of the method are given in Appendix S1.

Birds were captured in the funnel traps, except in one instance when downy chicks estimated to be seven days old were seen during a drive. One chick from this brood was captured by hand near where it was first detected, to reduce disturbance. The assumed location of this brood before disturbance was the actual capture location because chicks of this age move slowly in response to disturbance (Tyler *et al.* 1998), but in all other cases the brood location before disturbance occurred was taken to be the centre of the drive area. Although the locations of broods before the disturbance caused by the drive would have been distributed within the drive area, we took its centre to be a reasonable approximation of the mean of possible undisturbed positions when calculating the distance of chick locations to the singing place of their father. We assessed the sensitivity of our conclusions about chick-father distances to this assumption by measuring the shortest and longest distances between any part of the drive area in which a chick was captured and the father's singing place.

The age of captured young was estimated from measurements, using methods described in Appendix S1. The date of laying of the first egg of the clutch from which they hatched was estimated using the mean age of the brood and assuming 26 days between first egg and hatching date. Eight days is the laying period of a typical clutch and 18 days is the usual incubation period (Green *et al.* 1997b).

Buccal swab samples were collected as for singing males. Genomic DNA was extracted and genotyped for 15 microsatellite loci. Parentage assignment was performed from data for adults and young using methods described in Appendix S1.

RESULTS

In each study year, most (71–95%) of the singing male Corn Crakes present were captured and sampled (Table 1). Seventeen of the 43 males were captured more than once during the same breeding season to read the ring number and check their identity. Although most males were recorded as singing within a few hectares throughout the breeding season, some individuals moved up to 1.2 km. Movements exceeding 200 m were detected by recapture for 11 males (26%; Table 1). Microsatellite genotypes were obtained for all 43 of the sampled adult males and for five adult females captured during drives (Table 1).

Paternity was assigned to sampled fathers with a probability ≥ 0.80 for 16 chicks and six juveniles, which were assigned to 14 broods based on their estimated hatching dates (Table 2). Ten sampled adult males were assigned as fathers of captured young. Four of the fathers were each assigned two broods in the same breeding season (Table 2). In three cases, the two broods with the same father had different mothers (broods 1 and 2, 3 and 4, 9 and 10) and in one case the mother was the same for both broods (broods 6 and 7). The two broods with the same mother were captured on the same drive and had first-egg dates which differed by 34 days. Of the three pairs of broods with the same father, but different mothers, the first comprised two fledged juveniles captured on the same drive and the others were captured 1153 m and 168 m apart with first-egg dates 13 and 33 days apart. The locations of broods in relation to all of the recorded singing places of their assigned sires are mapped in Appendix S2.

Broods of chicks up to 20 days old, which would mostly still be dependent on the mother, tended to be close (median 78 m; range 4–151 m) to the singing location of the father, but older unfledged chicks, which would all be independent, were further away (median 261 m, range 149–601 m: Mann-Whitney *U*-test; $U_{3,7} = 1$, two-tailed $P = 0.034$; Fig. 1). However, there was no significant correlation overall between the distance from the father's singing place and chick age for unfledged chicks (Spearman's coefficient $r_S = 0.225$, one-tailed $P = 0.266$; $N = 10$). Distances of fledged juveniles from their father's singing location were similar to those of chicks older than 20 days (median 180 m; range 120–823 m; $U_{7,8} = 21$, two-tailed $P = 0.266$). The mean distance of all unfledged chicks from the father's singing place was 243 m (se ± 55 m) and the mean distance for fledged juveniles was 298 m (se ± 83 m).

We assessed the sensitivity of our conclusions about unfledged chick–father and juvenile–father distances to the uncertainty about where undisturbed chicks were located before drives began by using the closest and furthest possible locations of the brood, relative to the father's singing place, before it was disturbed by the capture process, instead of assuming that the undisturbed brood was at the centre of the drive area. As expected, the distances obtained from these extreme alternative assumptions were smaller and larger respectively than those obtained using the drive centres, but the results remained broadly similar. If we assumed that an unfledged chick was as close as it could possibly have been to its father, whilst being within the drive area, the mean distance was 163 m (range 0–451 m) and two of the ten observations still exceeded the threshold distance of 250 m previously considered to be safe (O'Brien *et al.* 2016). If it was assumed that an unfledged chick was as far as it could possibly be from its father, the mean distance was 356 m (range 78–724 m) and eight of the ten observations exceeded the 250 m threshold distance. For juveniles, the

equivalent mean distances for the closest possible and furthest possible alternative assumptions were 170 m (range 0–711 m) and 447 m (range 278–952 m) respectively.

For four broods, the father assigned to an unfledged brood was the male singing, at around the time of laying of the first egg, closer to the brood's first capture location than any other sampled male; for three broods the father was the second closest male; and, for one brood, it was the third closest male (Table 2). We refer to this relative ranking of the father, relative to other sampled males, as his distance rank. For the fathers of six young birds first captured as juveniles, the distance ranks were 1, 1, 2, 3, 5 and 6 (Table 2). The first location of every brood was much closer to the singing location nearest in time to the first egg dates of the male assigned as its father than the mean distance from the brood location of the singing places closest to that date of all the other sampled males in that year (Table 2). This tendency of broods to be closer to the singing location of the father, than the mean for other sampled males that were not the father, was highly significant (Wilcoxon matched-pairs signed ranks test, one-tailed $P < 0.005$).

Maternity was assigned to sampled mothers with a probability ≥ 0.80 for 18 chicks and three juveniles, which were assigned to seven broods based on their estimated hatching dates. All five sampled adult females were assigned as mothers. Two of the sampled females had two sampled broods in the same breeding season; both broods of one female were sired by the same male with first-egg date 34 days apart, and those of the other female were sired by two different males with first-egg dates 31 days apart.

DISCUSSION

Our results from DNA-based parentage assignment are consistent with those obtained from radio-tracking studies in finding evidence of some males fathering young with more than one female and of young with the same mother from two broods with hatching dates separated by approximately the expected time interval between first and second clutches. We also found that broods of chicks up to 20 days old were within 151 m of the singing location of the father at around the time of the first-egg date of the clutch, which is as expected from the radio-tracking determinations of locations of nests and dependent broods. However, independent unfledged chicks older than 20 days were located at least 149 m, and up to 601 m, from the singing place of their father, and fledged juveniles were up to 823 m away. Our findings were not affected by displacement or disturbance caused by mowing because no mowing had occurred within our study area at the time of drive catching. Guided by the radio-tracking results, the Corncrake Initiative, a conservation project operated by the RSPB, offered payments to farmers for voluntary adoption of delayed and Corn Crake-friendly mowing within 250 m of locations of singing males (O'Brien *et al.* 2006), but our study indicates that 40% of locations of all unfledged chicks were further away than this threshold distance, beyond which unmodified mowing has previously been considered safe. We propose that delayed mowing and Corn Crake-friendly mowing should therefore be deployed up to about 500 m from the singing places of adult males. This increase in distance from the previous recommendation of 250 m is intended to reduce the risk that flightless chicks independent of the mother are killed by mowing. Our results support previous finding that modifying mowing dates and methods within 250 m of male singing places is sufficient to reduce the risk that nests and dependent chicks are destroyed. Protection of fledged juvenile Corn Crakes

from mowing is less important because they can escape by flying and are rarely killed by mowing (Green *et al.* 1997b).

There are several potential sources of uncertainty in our estimates of brood–father distance and we assess the importance of these in Appendix S3. The largest source probably arises from our assumption that the unknown undisturbed locations of captured chicks were the centres of drive areas. We tested the robustness of our conclusions to this assumption by making extreme alternative assumptions about where young had been located within the drive areas before disturbance. Even when we assumed that every chick was as near as it could possibly have been to its father's singing location, one-fifth of unfledged chick locations were still more than 250 m away. We therefore suggest that the area within which mowing is considered to be safe for Corn Crake nests and unfledged chicks should be extended from 250 m to 500 m and that methods for the targeting of the location of agri-environment delivery within core areas for the species should adopt this rule.

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SUPPORTING INFORMATION

Appendix S1. Supplementary Methods.

Appendix S2. Maps of all recorded singing locations attributed to individual male Corn Crakes assigned as fathers of captured young.

Appendix S3. Assessment of the potential effects of uncertainty and failure of assumptions on the conclusions of the study.

LEGENDS TO FIGURES

Figure 1. Distances (m) between locations of Corn Crake broods captured as chicks (open circles) and as fully-grown juveniles (filled circles) and the singing location of their father on the date closest in time to the first-egg date of the clutch from which the brood hatched. Distances are plotted against the estimated age of the chicks or juveniles in days. Lines between symbols connect repeat observations of young from the same brood. The filled square and the vertical line through it show the mean and range respectively of the distance of nests of radio-tagged female Corn Crakes from the singing place of the male with which they mated (from Tyler & Green 1996).

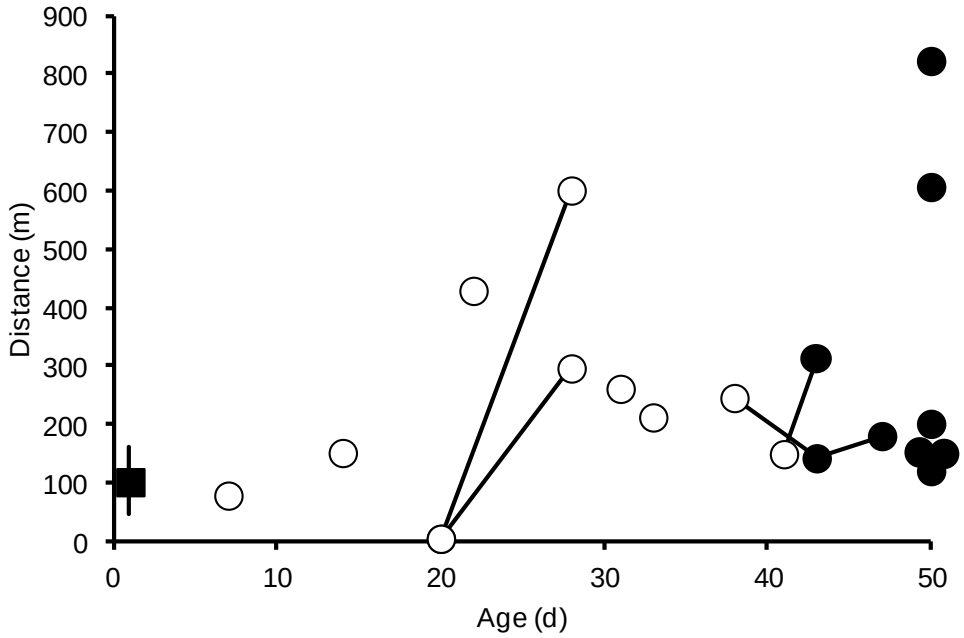
Table 1. Surveys and captures of singing male Corn Crakes and drive catching of adults, chicks and juveniles at the Nene Washes in 2013–2015.

Year	2013	2014	2015
Adult male surveys and captures			
Survey period	15 May - 18 July	30 April - 19 July	30 April - 9 July
Survey nights	27	26	24
Singing records	48	174	106
No. singing males	7	22	21
Largest count on 1 night	6	16	10
Date of largest count	26 May	18 June	25 May
Capture events	7	29	27
No. males captured	5	21	17
No. males captured twice or more	2	7	8
No. males moving > 200 m	2	4	5
Maximum movement (km)	1.2	1.0	0.5
Drive captures of adults, chicks and juveniles			
Drive period	1 August - 11 August	23 July - 21 August	26 July - 18 August
No. drives	7	18	8
No. chicks captured	18	8	1
No. juveniles captured	6	4	2
No. adult males captured	1	4	0
No. adult females captured	3	2	0

Table 2. Captures and recaptures of 14 broods of Corn Crake chicks and juveniles with fathers identified by microsatellite-based paternity assignment with probability ≥0.80. Brood numbers underlined have an assignment probability ≥0.90. Broods marked with asterisks in the age at capture and first-egg date columns were first captured as juveniles with fully-grown primary feathers, so their age estimate is approximate. The mean distance of the brood from non-fathers is the mean of distances from the capture location of the brood to the singing places, on the date nearest to the first-egg date of the clutch, of the DNA-sampled male Corn Crakes that were not the father of the brood. The distance rank is the rank distance from the brood location to the singing place of the father relative to that of the other sampled males in that year (i.e. 2/21 means that the father's singing location at the date closest in time to the brood's first-egg date was the second closest to the brood location of the 21 males sampled). These two measures are only shown for the first capture of each brood. The first-egg dates are given as days elapsed after 31 December of the previous year.

Year	Brood code	Brood members	Brood members captured	Father	Brood age at capture (days)	First-egg date	Distance of brood from father's singing place (m)	Mean distance from non-father's singing places (m)	Distance rank of father's place
2013	1	EY11035	EY11035	EG59372	50*	138*	148	1505	1/5
2013	2	EY11036	EY11036	EG59372	50*	138*	148	1505	1/5
2013	3	EY11034	EY11034	EG59373	31	155	261	1632	1/5
2013	4	EY11041, 42, 45, 64	EY11041, 42, 45	EG59373	20	168	4	1068	1/5
2013	4	EY11041, 42, 45, 64	EY11045	EG59373	28	168	296	-	-
2013	4	EY11041, 42, 45, 64	EY11064	EG59373	28	168	601	-	-
2014	5	EY11304	EY11304	EY11058	50*	130*	201	1938	2/21
2014	6	EY11301, 02, 03	EY11301, 02	EY11114	41	137	149	1858	1/21
2014	6	EY11301, 02, 03	EY11303	EY11114	43	137	312	-	-
2014	7	S102	S102	EY11114	7	171	78	1868	1/21
2014	8	EY11287	EY11287	EY11152	50*	148*	823	1848	6/21
2014	9	EY11263, 64, 86	EY11263, 64	DE32711	38	151	244	1829	2/21
2014	9	EY11263, 64, 86	EY11286	DE32711	43	151	142	-	-
2014	9	EY11263, 64, 86	EY11263	DE32711	47	151	180	-	-
2014	10	EY11289, 90	EY11289, 90	DE32711	14	184	151	1929	2/21
2014	11	EY11285	EY11285	EY11034	22	171	429	1834	2/21
2015	12	EY11445	EY11445	EY11381	50*	131*	607	1318	5/17
2015	13	EY11455	EY11455	EY11110	50*	136*	120	1090	3/17
2015	14	EY11444	EY11444	EY11251	33	148	212	1484	3/17

Figure 1. Distances (m) between locations of corncrake broods captured as chicks (open circles) and as fully-grown juveniles (filled circles) and the singing location of their father on the date closest in time to the first-egg date of the clutch from which the brood hatched. Distances are plotted against the estimated age of the chicks or juveniles in days. Lines between symbols connect repeat observations of young from the same brood. The filled square and the vertical line through it show the mean and range respectively of the distance of nests of radio-tagged female corncrakes from the singing place of the male with which they mated from Tyler & Green (1996).



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APPENDIX S1

SUPPLEMENTARY METHODS

Study area

The Nene Washes (52.58°N, 0.07°W) is a canalised section of the River Nene in Cambridgeshire, England, UK. It was built as a flood protection structure during the drainage of the marshlands of the Fenland Basin. The 15 km^2 strip of wet grassland, up to about 1 km wide, parallel to the river, is designated as a Site of Special Scientific Interest under the Wildlife and Countryside Act 1981 because of its aggregations of breeding and non-breeding birds and its ditch and grassland flora. It is also a site designated under the Ramsar Convention on Wetlands, a Special Area of Conservation under Article 3 of the European Union's Habitats Directive and a Special Protection Area under Article 4 of the European Union's Birds Directive. Corn Crakes ceased to breed at the site early in the 20th Century. Part of the site is owned and managed as a nature reserve by the RSPB. Since 2004, the Nene Washes has a re-introduced breeding population of Corn Crakes located in a section of the grassland strip about 7 km long.

Singing male survey and capture methods and interpretation

Adult male Corn Crakes produce a loud rasping 'crek-crek' song almost continuously on most nights when they are not in a short-term (7–10 days) pair bond with a female (Tyler & Green 1996). Survey routes, traversed using a vehicle, were planned to approach within 300 m of all areas of tall grass and herbs potentially suitable for Corn Crakes. Surveys were conducted between 22:00 and 03:00 BST at intervals of 2 – 4 days on nights with suitable weather

conditions. Rain and windy conditions (Beaufort Force 5 or more) were avoided. Co-ordinates of the locations of singing males were first identified by triangulating the sound from mapped listening points. Singing places were later approached on foot to within 50-100 m and their locations determined using the mapped locations of features such as ditches, bushes and gateways and a hand-held GPS (Garmin eTrex 10). All of the singing locations used to calculate distances between capture localities of young Corn Crakes and the singing sites of their potential fathers were known to within 20 m.

Night-time records of singing males were assigned to individuals using their locations and whether or not sets of males were recorded singing on the same night. We began the process of assigning records to individuals by identifying as separate individuals males that were singing on the same night, beginning with the night when the maximum number was counted. The presence of individuals additional to this set was identified by capturing them to read or apply BTO rings.

Singing males were captured at night by luring them into nearby mistnets using a broadcast recording of conspecific song. Their capture locations were determined using a hand-held GPS. In the absence of capture evidence to the contrary, we assumed that night-time singing records within 200 m of a capture location were of the ringed individual caught there, but we often made further captures to check this. We attempted to capture all the males detected on night-time surveys and, in doing this, we captured some males more than once, with 14 being captured twice and three on three occasions during the course of the same breeding season. Recaptures usually occurred when a singing male was heard in an area where no male had been caught previously in the season, but capture revealed that it was an individual already captured elsewhere which had moved. A few males evaded repeated attempts at capture throughout the breeding season, but we consider that these were

identifiable as unique and separate individuals with reasonable confidence, based upon their evasion behaviour and locations.

We were unable to estimate the number of adult females present at the Nene Washes because there is no method available for surveying them. However, we note that equal numbers of adult males and adult females were captured on July–August drives (Main text: Table 1), so the number of adult females in the population was probably similar to the counts of singing males.

Validation of estimated numbers of singing males

The accuracy of our assessment of the total number of singing males present depends upon whether we correctly identified as separate individuals the males we were unable to catch whilst they were singing. To check this we performed a mark-recapture analysis of data collected by the same methods as those described here, but obtained over a longer period (2004–2018) than is considered in this paper. From all ringing and recapture records of adult males from this period, we identified the Manly-Parr set of observations (Manly & Parr 1968), each of which refers to a male-year in which an individual was known to be alive because it had been recorded in a previous year and also in a subsequent year. The Manly-Parr set comprised 16 male-years involving 15 males in 6 focal years (2008, 2009, 2010, 2014, 2016 and 2017). The male was captured whilst singing in 15 of the 16 male-years (annual probability of capture = 15/16 = 0.938, binomial confidence limits, 0.698 – 0.998). If our method for assessing the total number of singing males is accurate, we would expect that this annual probability of capture derived from mark-recapture analysis present would be the same as the ratio of the number of individuals captured to the total estimated present. For the six Manly-Parr focal years, the mean of the ratio of the number of males captured to the total estimated was 0.824

(95% confidence limits, 0.651 – 0.997). This analysis indicates that the ratio of minimum number of individuals known present from captures to our estimates of the number of singing males present was similar to, and not significantly different from, the expectation based upon the mark-recapture estimate of the annual probability of capture. In our study period, all five of the adult males captured during drives in July–August, after the end of the singing season, had already been captured earlier in the same year as singing males (Main text Table 1). Combining both of these lines of evidence, we are confident that we captured and sampled a high proportion of the potential fathers of the chicks we sampled.

Drive catching and sampling of adults, chicks and juveniles

Corn Crake adults, chicks and juveniles were captured by driving them into funnel traps, similar to Ottenby traps (Bub 1991), made from flexible plastic netting (Cintoflex M, Tenax UK Ltd, Wrexham, UK). For each drive, an approximately rectangular area of 1.2–4.7 ha of tall grass and herbage was enclosed by a combination of fences of plastic netting and existing barriers, such as water-filled ditches. Corn Crakes within it were driven into a line of traps set approximately equally spaced at one end of the drive area and linked by drift fences. Further details of the method are given by Green (2010).

A slow (<200 m/h) drive was made by a team of people towards the trap-line from the opposite end of the drive area, using tractor noise generated by MP3 players and disturbance of the ground vegetation by dragging a 2.5 cm diameter polypropylene rope over it. In one instance, when downy chicks estimated to be seven days old were seen and heard calling during a drive, the number in the brood was estimated by eye and only one was captured by hand, to reduce disturbance. Traps were checked periodically and the captured birds were placed in cloth bags. The assumed location before disturbance of the young chick captured

by hand was the actual capture location because chicks as young as this move slowly in response to disturbance (Tyler *et al.* 1998) and produce loud calls when separated from their mother (Green *et al.* 1997b). In all other cases, the brood location before disturbance occurred was taken to be the centre of the drive area. Although the true locations of broods before the disturbance caused by the drive would probably have been more uniformly distributed within the drive area than this, we assumed that the centre of the drive area was a reasonable approximation of the mean of undisturbed positions when calculating the distance of chick locations to the singing place of their father. However, to assess the sensitivity of our conclusions about the chick-father distances to failure of this assumption, we also measured the shortest and longest distances between any part of the drive area in which a chick was captured and the father's singing place. Further details of the catching method are given by Green (2010). The age of captured young was estimated from measurements, using established methods described below. Buccal swab samples were collected. Chicks and juveniles were released in the drive area close to the trap in which they were caught. Where probable mothers were caught with young, they were released together.

Determining the age of chicks

Captured chicks and juveniles were distinguished from adults following Salzer & Schäffer (1997). All birds, except the chick of seven days old, were marked with uniquely numbered BTO metal rings. Body weight, maximum chord wing length and the length of the waxy sheath on the growing 7th primary (numbering descendantly from proximal to distal) were measured Green & Tyler (2005). Young of the year, with no waxy sheath on the 7th primary, were classed as fully-grown juveniles. Other young were classed as unfledged chicks. The age of chicks weighing less than 109 g was estimated from the body weight and that of heavier

chicks from the ratio of the length of the waxy sheath to the maximum chord wing length, by the method of Green & Tyler (2005).

The hatching date of a group of chicks of similar age (< 3 days different), identified as siblings from the microsatellite results, was estimated by subtracting the mean age of the brood from the capture date. Fully-grown juveniles, not captured previously as chicks, could not be aged using body weight or primary wax, so we assumed that they were 50 days old because primary growth is completed at 45 days old (Green & Tyler 2005) and radio-tagged juveniles have been found to depart from the natal area soon after this (Donaghy *et al.* 2011). The first-egg date of the clutch from which a brood was derived was taken to be 26 days before the hatching date, assuming eight days as the laying period of a typical clutch and 18 days as the incubation period (Green *et al.* 1997b).

DNA sampling and extraction and parentage assignment

The mouth of each captured bird was swabbed using a sterile cotton swab on a wooden stick (Sterilin F150CA) rotated gently against the buccal epithelium anterior to the base of the tongue 20–30 times. The swab was then replaced in its plastic protective sheath. Within a few hours of sampling, the cotton bud was cut off the stick and stored in a tube containing sufficient 100% ethanol to immerse the bud.

DNA preparation and genotyping

Genomic DNA was extracted from the buccal swabs using an ammonium acetate method (Richardson *et al.* 2001). The DNA samples were then genotyped for 15 microsatellite loci (Gautschi *et al.* 2002, Brede *et al.* 2010, Dawson *et al.* 2010) and one sex marker (Dawson *et al.* 2015), which were run in three multiplex groups (Table S1). Polymerase chain reactions (PCR)

were run in a total volume of 10 μl, which contained: 5 μl multiplex PCR mix (Qiagen Inc., Valencia, USA), 2 μl ddH_2O, 1 μl fluorescently labeled primer-mix and 2 μl extracted DNA. The PCR program (Veriti Thermal Cycler - Applied Biosystems) was: 95°C for 15 min, then 40 cycles of 94°C for 30 s, 55°C for 90 s, and 72°C for 60 s, followed by a final 60°C for 30 min. Fluorescent-labeled PCR products were analyzed on a 3730 DNA Analyzer (Applied Biosystem, California, USA), and allele sizes were scored using GENEMAPPER 4.0 (Applied Biosystems) and a GeneScan 500 ROX size-marker (Applied Biosciences).

Parentage assignment

We assigned parentage first using a Bayesian approach, in R 3.2.2 (R Core Team 2017), using the package *MASTERBAYES* 2.52 Hadfield *et al.* 2006) and then in COLONY 2.0.3.3 (Wang 2013). We used 14 microsatellites; we excluded *Crex11* as some samples showed three peaks using GENEMAPPER, and this marker had high null allele frequencies in other Corn Crake populations (Fourcade *et al.* 2016).

We tested for Hardy-Weinberg Equilibrium (HWE) and Linkage Disequilibrium (LD) using GENEPOP 4.2 (Rousset 2008). For the HWE and LD analyses, we removed potential relatives using COANCESTRY 1.0.1.7 (Wang 2011) to choose pairs with zero relatedness based on TrioML. We selected individuals that had the highest number of zero relatedness (≥18) with other individuals in the populations, which resulted in 31 individuals.

Seven loci deviated from HWE: *Crex6*, *Crex8*, *Crex12*, *N3B3*, *Crex2*, *TG02-120*, and *TG04-012*. These deviations may arise from the small dataset (N=31) and the presence of some relatives. We therefore also conducted HWE tests on the same 14 loci from 28 captive bred Corn Crakes, from which birds introduced into the Nene Washes population originated. Four loci deviated from HWE (*Crex6*, *Crex1*, *Crex2* and *TG04-012*) and only two of these were the

same as in the Nene Washes population. Furthermore, of 210 HWE tests from 15 Corn Crake populations using the same loci, (except for *N3B3*), 23 deviated from HWE (Fourcade *et al.* 2016), but the loci differed across populations.

After False Discovery Rate (FDR) control (Benjamini & Hachberg 1995), to account for multiple tests, four pairs of loci were in LD: *Crex8* & *TG04*-041, *TG04-041* & *TG12-015*, *Crex9* & *TG04-012* and *TG12-015* & *TG05-030*. We also conducted LD tests on the captive bred birds; after FDR control, two pairs of loci were in LD (*Crex6* & *Crex8*, and *Crex8* & *TG12-015*), but these differed to those in the wild population. No deviations from LD were detected across 15 populations using the same loci, (except for *N3B3*) by a previous study (Fourcade *et al.* 2016), so all 14 loci were retained in our analyses.

Parentage was assigned in *MASTERBAYES* using allele frequencies extracted from all 81 genotyped birds from the Nene Washes population, and a default allelic drop-out and stochastic error rate of 0.005. The number of unsampled mothers and fathers were estimated by *MASTERBAYES* and no restrictions were placed on the number of tolerated mismatches between parents and offspring. Paternity assignments were weighted by the Euclidian distance between the candidate father (N = 43) and offspring (N = 31), and both parents were sampled simultaneously. Maternity assignments were not weighted by distance as only five adult females were genotyped and models containing this parameter did not converge. We ran 130,000 iterations, saving every 100th and discarding the first 30,000, to ensure autocorrelations between successive parameter estimates were <0.1. Metropolis-Hastings acceptance rates were checked to lie between the acceptable range of 0.2 and 0.5 (Hadfield *et al.* 2006).

We then assigned additional sibships for offspring born in 2013 and 2014, using COLONY. We specified the parents already assigned with a probability ≥0.80 from

MASTERBAYES. We assumed monogamy for males and females, a probability of 0.2 of either the mother of father being in the candidate mother or father pools, and an error rate of 0.01.

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Table S1. Details of the three multiplexes (MP) used to analyse the 15 microsatellite loci and one sexing locus, along with their fluorescent label, primer concentrations and reference sources.

Locus	MP	Fluorescent label	Primer concentration in primermix μM)	Source
Crex6	1	Hex	0.5	Gautschi *et al.* (2002)
Crex9	1	Ned	0.5	Gautschi *et al.* (2002)
Crex7	1	Fam	0.5	Gautschi *et al.* (2002)
TG04-041	1	Hex	0.25	Dawson *et al.* (2010)
Crex8	1	Fam	0.5	Gautschi *et al.* (2002)
TG012-015	1	Hex	7.5	Dawson *et al.* (2010)
Z37B_sex	2	Fam	0.25	Dawson *et al.* (2015)
Crex11	2	Hex	0.5	Gautschi *et al.* (2002)
Crex12	2	Ned	0.5	Gautschi *et al.* (2002)
N3B3	2	Fam	0.5	Brede *et al.* (2010)
TG04-012a	2	Hex	0.5	Dawson *et al.* (2010)
Crex2	3	Hex	0.5	Gautschi *et al.* (2002)
TG04-012	3	Ned	0.5	Dawson *et al.* (2010)
Crex1	3	Fam	1.5	Gautschi *et al.* (2002)
TG05-030	3	Hex	0.5	Dawson *et al.* (2010)
TG02-120	3	Fam	0.5	Dawson *et al.* (2010)

APPENDIX S2

Maps of all recorded singing locations attributed to individual male Corn Crakes assigned as fathers of captured young

Figure S1. Map of part of the study area showing all night-time singing locations (diamonds) attributed to adult male Corn Crake EG59372 in 2013. Numerals beside the diamonds show the Julian date (1 January = 1) of each observation. The male was captured to check his ring number at the singing location for which the Julian date is boxed. Capture localities (shaded squares) are shown for two fledged juveniles with different mothers of which EG59372 was the father (broods 1 and 2 in Table 2). The shaded diamond shows the singing place closest in time to the first-egg dates of both juveniles. Co-ordinate labels show northings and eastings in metres in Ordnance Survey square TL. The grid consists of 200-m squares. In these diagrams the shaded triangles, circles and squares denote the age of the brood at capture: triangles identify broods 20 days of age or younger, circles identify older unfledged chicks and squares identify fledged juveniles.

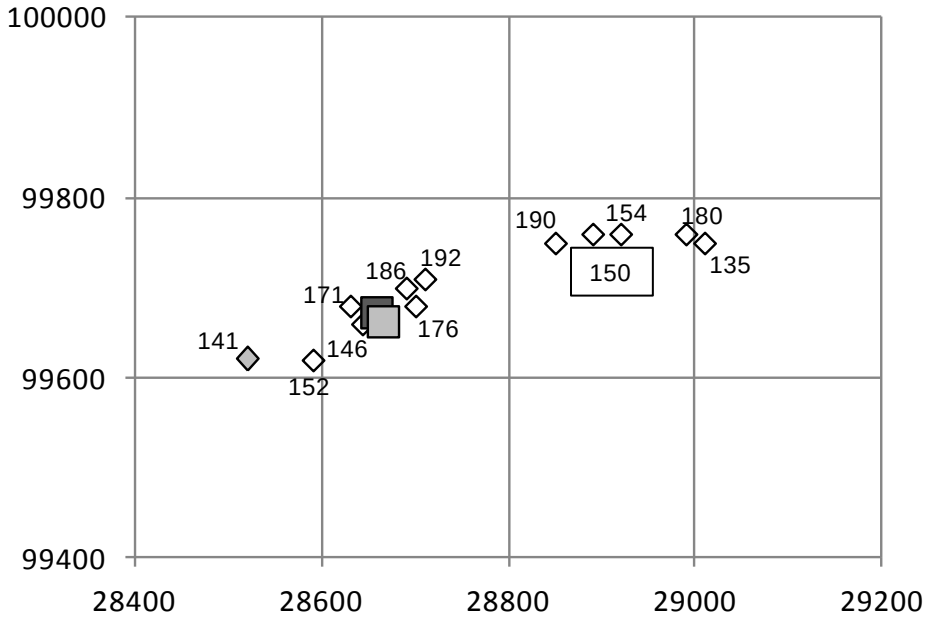

Figure S2. Map of part of the study area showing all night-time singing locations (diamonds) attributed to adult male Corn Crake EG59373 in 2013. Numerals beside the diamonds show the Julian date (1 January = 1) of each observation. The male was captured to check his ring number at the two singing locations for which the Julian date is boxed. Capture localities are shown for a chick (light grey circle) from brood 3 and three captures of chicks from brood 4 (dark grey triangle and circles) (see Table 2) of which this male was the father. The shaded diamonds show the singing places closest in time to the first-egg dates of the two broods, with the shading identifying singing places associated with each brood. Other conventions are as in Figure S1.

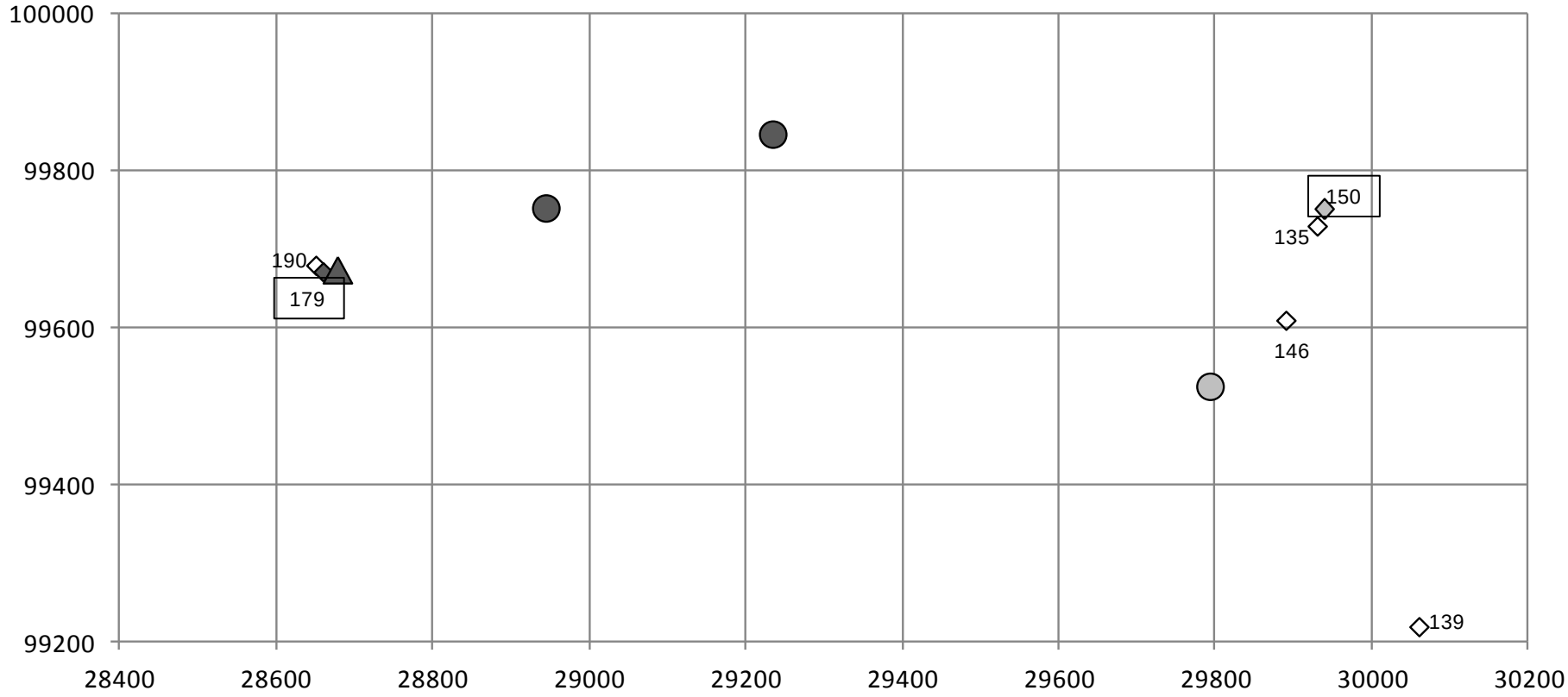

Figure S3. Map of part of the study area showing all night-time singing locations (diamonds) attributed to adult male Corn Crake EY11058 in 2014. Numerals beside the diamonds show the Julian date (1 January = 1) of each observation. The male was captured to check his ring number at the singing location for which the Julian date is boxed. Capture localities are shown for a juvenile (light grey square) from brood 5 (see Table 2) of which this male was the father. The shaded diamond shows the singing place closest in time to the first-egg date of the brood. Other conventions are as in Figure S1.

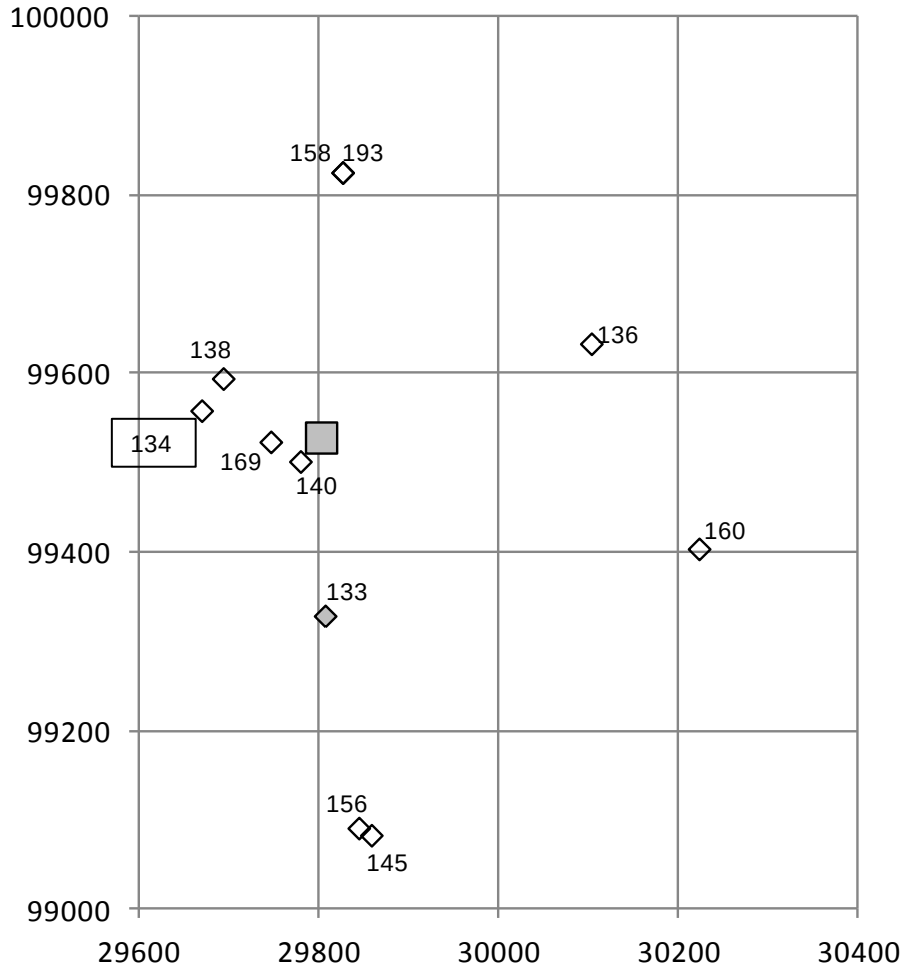

Figure S4. Map of part of the study area showing all night-time singing locations (diamonds) attributed to adult male Corn Crake EY11114 in 2014. Numerals beside the diamonds show the Julian date (1 January = 1) of each observation. The male was captured to check his ring number at the two singing locations for which the Julian date is boxed. Capture localities are shown for chicks and a juvenile (light grey circle and square) from brood 6 and chicks from brood 7 (dark grey triangle) (see Table 2) of which this male was the father. The shaded diamonds show the singing places closest in time to the first-egg dates of the two broods, with the shading identifying singing places associated with each brood. Other conventions are as in Figure S1.

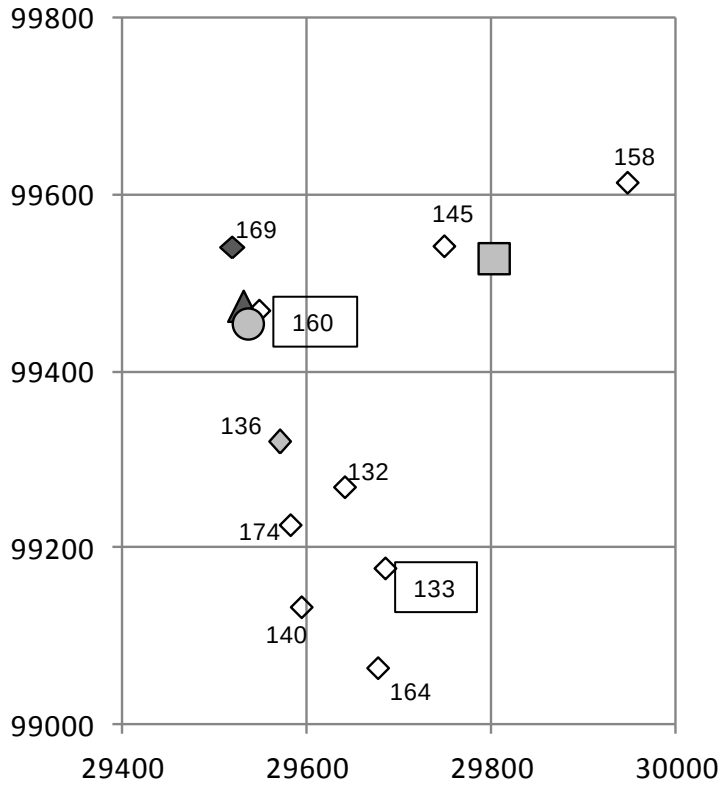

Figure S5. Map of part of the study area showing all night-time singing locations (diamonds) attributed to adult male Corn Crake EY11152 in 2014. Numerals beside the diamonds show the Julian date (1 January = 1) of each observation. The male was captured to check his ring number at the two singing locations for which the Julian date is boxed. Capture localities are shown for a juvenile (light grey square) from brood 8 (see Table 2) of which this male was the father. The shaded diamond shows the singing place closest in time to the first-egg date of the brood. Other conventions are as in Figure S1.

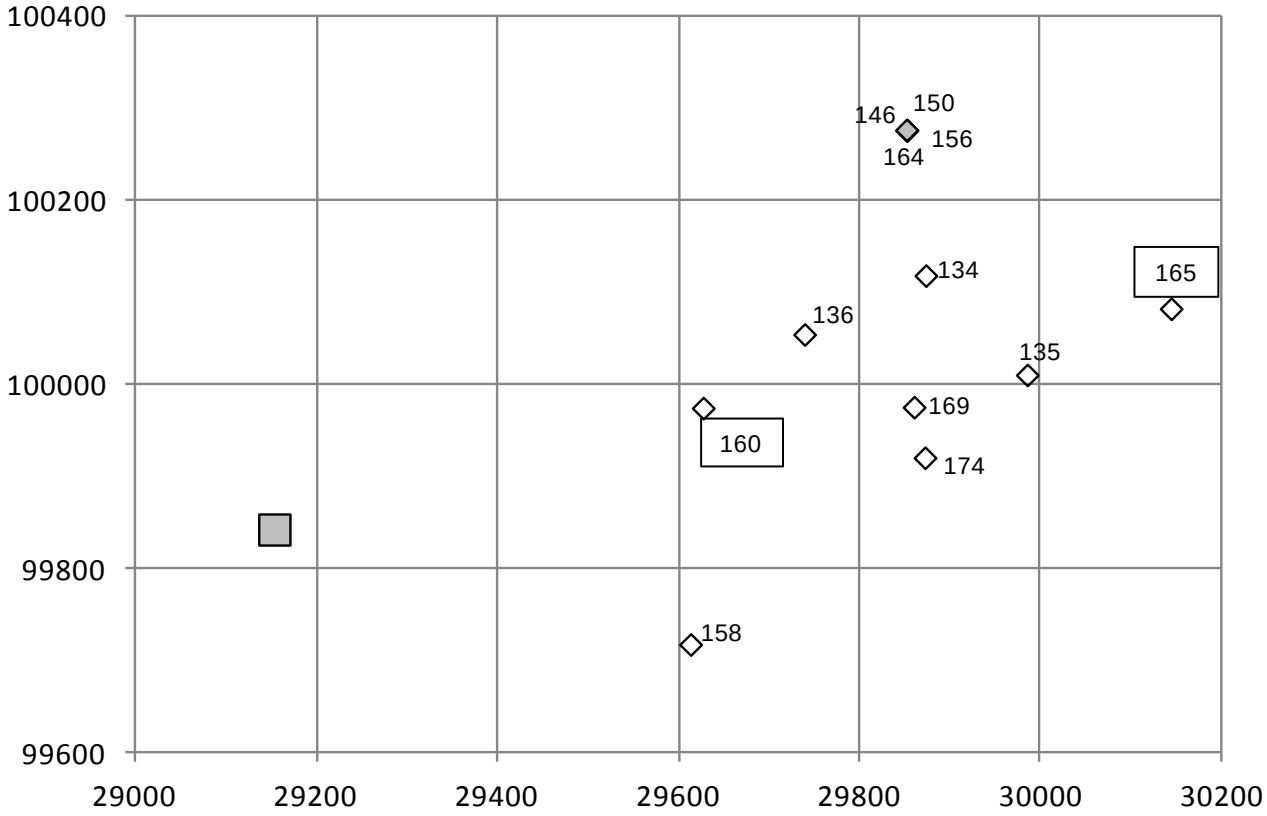

Figure S6. Map of part of the study area showing all night-time singing locations (diamonds) attributed to adult male Corn Crake DE32711 in 2014. Numerals beside the diamonds show the Julian date (1 January = 1) of each observation. The male was captured to check his ring number at the singing location for which the Julian date is boxed. Capture localities are shown for chicks and a juvenile (light grey circle and square) from brood 9 and chicks from brood 10 (dark grey triangle) (see Table 2) of which this male was the father. The shaded diamonds show the singing places closest in time to the first-egg dates of the two broods, with the shading identifying singing places associated with each brood. Other conventions are as in Figure S1.

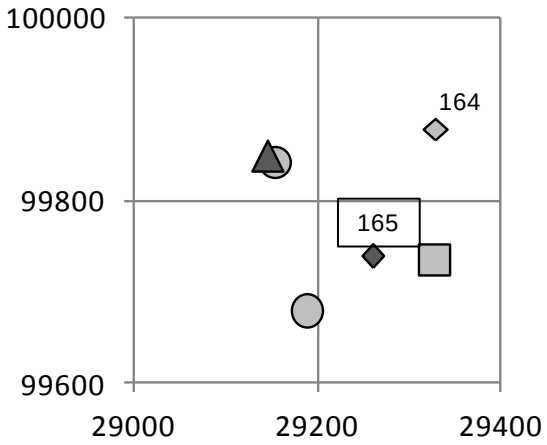

Figure S7. Map of part of the study area showing all night-time singing locations (diamonds) attributed to adult male Corn Crake EY11034 in 2014. Numerals beside the diamonds show the Julian date (1 January = 1) of each observation. The male was captured to check his ring number at the singing location for which the Julian date is boxed. The capture localities are shown for a chick (light grey circle) from brood 11 (see Table 2) of which this male was the father. The shaded diamond shows the singing place closest in time to the first-egg date of the brood. Other conventions are as in Figure S1.

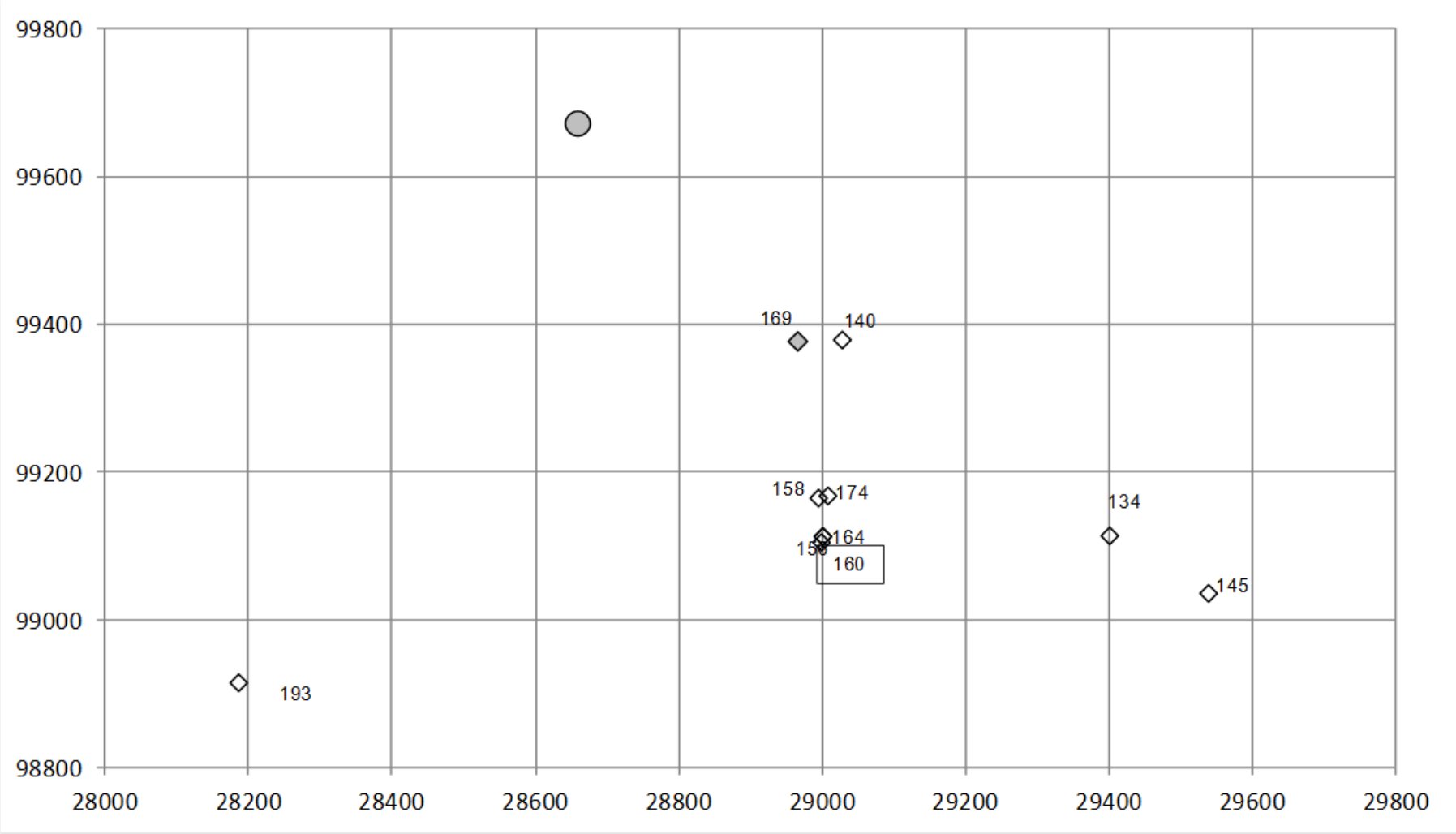

Figure S8. Map of part of the study area showing all night-time singing locations (diamonds) attributed to adult male Corn Crake EY11381 in 2015. Numerals beside the diamonds show the Julian date (1 January = 1) of each observation. The male was captured to check his ring number at the singing location for which the Julian date is boxed. The capture localities are shown for a juvenile (light grey square) from brood 12 (see Table 2) of which this male was the father. The shaded diamond shows the singing place closest in time to the first-egg date of the brood. Other conventions are as in Figure S1.

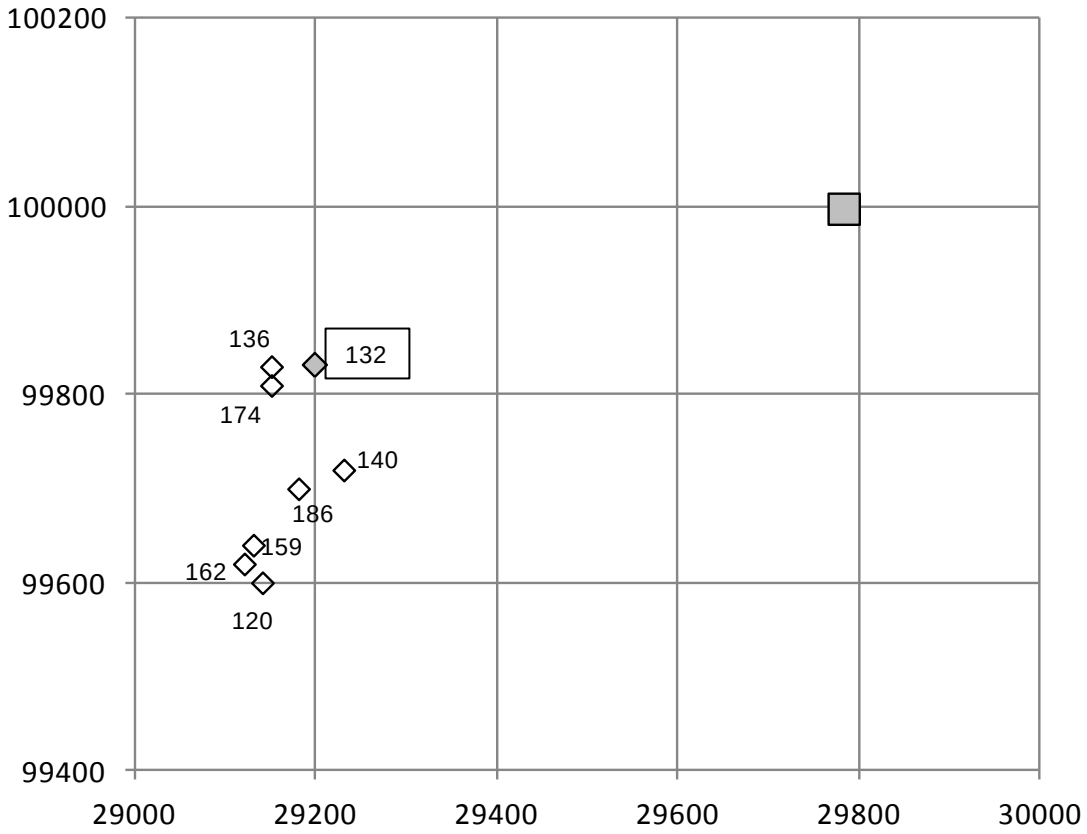

Figure S9. Maps of parts of the study area showing all night-time singing locations (diamonds) attributed to adult male Corn Crakes EY11110 (left) and EY11251 (right) in 2015. Numerals beside the diamonds show the Julian date (1 January = 1) of each observation. The males were captured to check their ring numbers at the singing locations for which the Julian dates are boxed. The capture locality is shown for a juvenile (light grey square) from brood 13 of which EY11110 was the father and a chick (light grey circle) from brood 14 of which EY11251 was the father (see Table 2). The shaded diamonds show the singing places closest in time to the first-egg date of these broods. Other conventions are as in Figure S1.

APPENDIX S3

ASSESSMENT OF THE POTENTIAL EFFECTS OF UNCERTAINTY AND FAILURE OF ASSUMPTIONS ON THE CONCLUSIONS OF THE STUDY

Principal sources of uncertainty

We identified the following potential sources of uncertainty which might affect the robustness for our conclusions as they apply to conservation practice.

(1) Difference between the location of a brood before the disturbance involved in drive catching and the assumed location at the centre of the drive area.

(2) Uncertainty about paternity assignment.

(3) Error in estimating the position of singing males.

(4) Uncertainty about the identity of the male at a singing location.

(5) Error in the identification of the singing location on or about the time of clutch initiation.

(6) Difference between ecological conditions in the study area and the main UK range of the Corn Crake in Scotland where most conservation practice might be affected.

We consider each of these in turn in the following sections.

Difference between the location of a brood before the disturbance involved in drive catching and the assumed location at the centre of the drive area

We report a simple assessment of the sensitivity of our conclusions to the assumption about brood location in the main text by repeating our analyses with broods assumed to be as near to and as far from the assigned father as possible, within the drive area where the brood was captured. We found that some broods were further from the father than the 250 m threshold

beyond which mowing was formerly thought to be safe even when we adopted the extreme assumption that all broods were located at the nearest point to the father before being disturbed by the drive. We consider that is highly unlikely that this extreme scenario is close to the real situation. Hence, we think that our conclusion is robust against failure of assumption locating broods at the drive centre.

Uncertainty about paternity assignment

Paternity of young was assigned to a particular sampled adult male if the estimated probability of paternity was ≥0.80. Whilst it is possible that another male was the father, it is unlikely that any of the other sampled males present in the hatching year was the father because the highest paternity probability for the highest ranking alternative sampled potential father was >0.20 lower in all cases. We also note that the distance rank of the assigned father of unfledged young was most frequently the nearest sampled male to the brood and never more than the third ranked male (see Main text and Table 2). This relative proximity, compared with other potential fathers, would be unlikely to occur if there were errors in paternity assignment. Finally, we would expect that, if paternity assignment errors occurred, they would be more likely for brood-father assignments with paternity probabilities between 0.80 and 0.90 than for assignments with probabilities ≥0.90. In that case, we would expect the distance ranks of assigned fathers to be lower (i.e. closer) for the brood-father assignments with probabilities ≥0.90. However, the mean distance rank for broods with paternity probabilities ≥0.90 was slightly higher (father further away: mean rank = 2.4; range 1-5; $N = 8$) than for those with paternity probabilities between 0.80 and 0.90 (mean rank = 2.0; range 1-6; $N = 6$). We conclude that errors in paternity assignment are unlikely to have occurred and are therefore unlikely to affect the robustness of our conclusions.

Error in estimating the position of singing males

We determined the singing positions at night, but singing male Corn Crakes can be approached to within about 10 metres without disturbing them and we approached on foot to check locations and used hand-held GPS devices and landmarks such as ditches and gateways (see main text Methods) to map positions. Six of the singing places of assigned fathers on the date nearest to the clutch initiation date involved capturing the male by placing a mistnet close to (< 10 m) the singing place. We consider that the accuracy of location of all the singing places of assigned fathers on the date nearest to the clutch initiation date was within 20 m. This distance is small compared to the mean brood-father distance, so we conclude that this potential source of error is unlikely to have biased our results significantly.

Uncertainty about the identity of the male at a singing location

We captured the male and applied or read his ring for six of the singing places of assigned fathers on the date nearest to the clutch initiation date, so there is no doubt about the identity of the male singing at that location in those cases. For the remaining cases we used the rules described in the main text of the Methods to infer which male was present at the singing site. Whilst we cannot exclude the possibility that a singing record was attributed the wrong male, we think that this is highly unlikely, based upon experience of capturing much larger numbers of singing males in areas of Scotland where the same record attribution rules were used. Another way in which such errors could have occurred would be if our method for determining the total number of singing males present from the night-time survey results had been inaccurate and had led to fewer males being assumed present than were really there. In that case, one of the 'missed' males could actually have been present at a singing location

which we attributed to a sampled male. However, the section of Appendix S1 'Validation of estimated numbers of singing males' indicates that our estimates of total numbers of males were not in error to any substantial extent. Hence, we conclude that errors caused by misattributing singing records to sampled individual males are unlikely to have occurred and are therefore unlikely to affect the robustness of our conclusions.

Error in the identification of the singing location on or about the time of clutch initiation

We calculated the probable clutch initiation date of a brood using estimates of chick age and calculated brood-father distances using the singing record closest in time to that date. The methods used to make the age estimates are accurate to within a few days when applied to a single young bird. In our study, we used measurements from up to four young to determine the average age of brood-mates in unfledged broods and this is likely to likely to have further increased accuracy. However, the nearest date of an available singing record of the assigned father of a brood to its estimated initiation date might well differ from the brood's true clutch initiation date by up to a few days. This might sometimes lead to the estimated brood-father distance being larger or smaller than the true distance. We do not think that there is a straightforward formal way to quantify the potential magnitude of such errors. However, inspection of the maps in Appendix S2 reveals three instances in which the location of an unfledged brood is more than 250 m from any of the singing locations of the assigned father within a ten day period centred on the clutch initiation date. We also note that our results are intended to be applied to practical conservation management in which managers decide where to offer protection from mowing risk in relation to locations of singing males recorded on their night-time surveys. They usually conduct two or three surveys per season compared with the 24-27 surveys conducted per season in our study. Therefore, many of the night-time

singing locations known to conservation managers, being many fewer per male, are likely, by chance, to be much nearer or much further from the position of the nearest brood than the distribution of true brood-father distances, if they were known. For this reason, our proposal to extend protection from mowing risk to areas within 500 m of the nearest singing male is not likely to lead to frequent erroneous and unnecessary protection of areas where broods are absent. Taking all these factors into account, we conclude that error in the identification of the singing location on or about the time of clutch initiation is unlikely to affect the robustness of our conclusions about conservation management.

Difference between ecological conditions in the study area and the main UK range of the Corn Crake in Scotland where most conservation practice might be affected.

Ecological conditions within the range of the Corn Crake in Scotland vary substantially from area to area, as do Corn Crake population densities. Hence, we cannot exclude the possibility that brood-father distances in some areas of Scotland are larger or smaller than those we estimated at the Nene Washes. However, we note that the brood-father distances for broods up to 20 days old were as was expected from the results from previous radio-tracking studies of nest locations and dependent brood locations in Scotland. This makes a large discrepancy due to our study site being in southern England rather than Scotland unlikely. In several respects, the Nene Washes grasslands are broadly similar to those in many parts of the Corn Crake's range in Scotland. They are divided up into fields by ditches and some fields are grazed by livestock so that they have vegetation too short for Corn Crakes, whilst others have livestock excluded to produce hay or silage crops so the vegetation is sufficiently tall. If anything, the Nene Washes grasslands have a greater proportion with tall enough vegetation for Corn Crakes than most parts of the Corn Crake range in Scotland, principally because

grass growth is more rapid in the south. This would lead us to expect that Corn Crake broods might need to move less far from the nest to find safe foraging areas at the Nene Washes. If that was the case, the safe distances between singing male sites and areas of mowed grassland might need to be larger in Scotland than the 500 m we suggest based upon our study in England. However, given that we are already proposing that the threshold distance should be doubled, we suggest that evidence from further research in Scotland would be needed before adoption of a larger safe distance would be justified.