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## Sexual selection and reproductive strategies in songbirds

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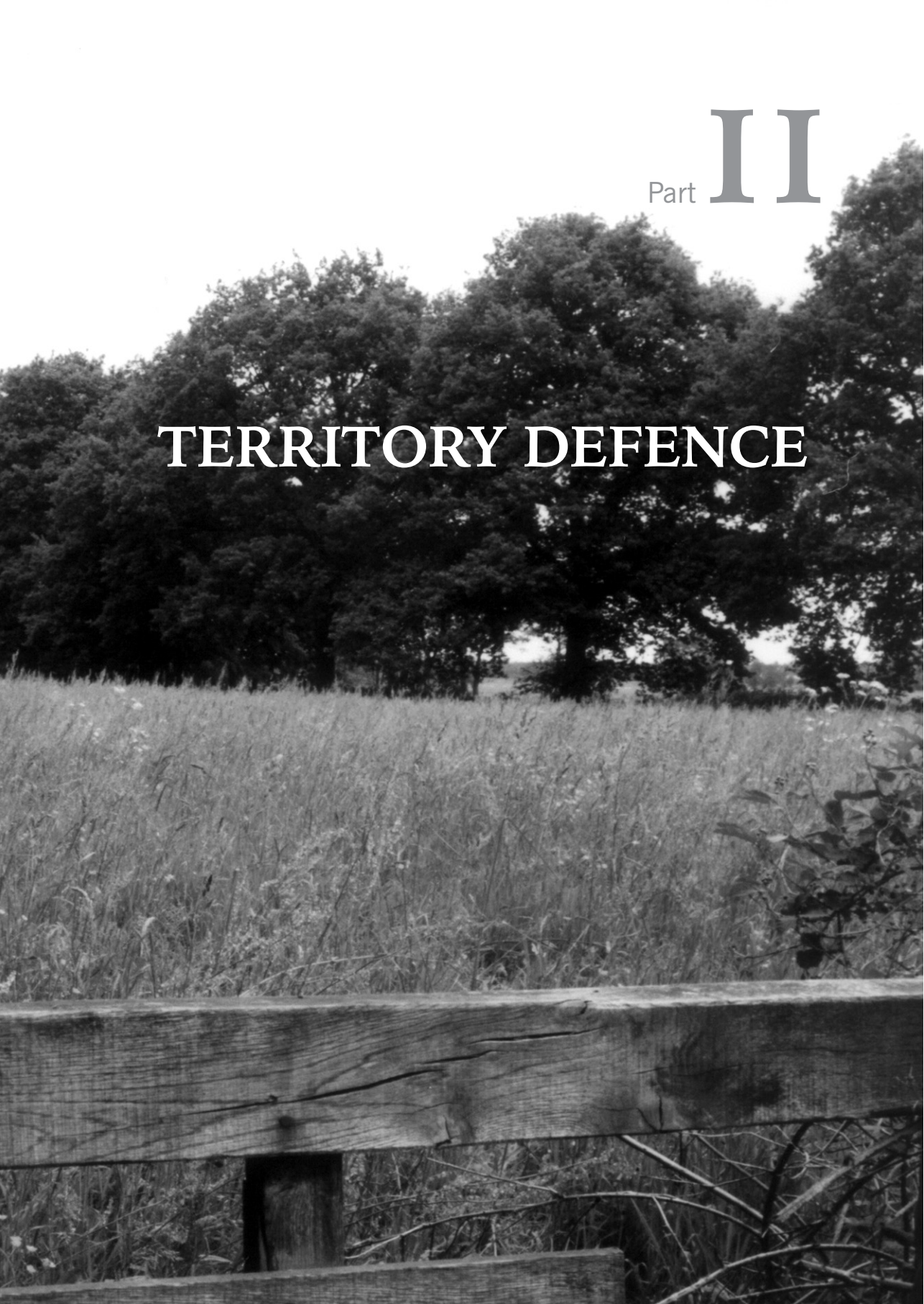
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Part **II**

# TERRITORY DEFENCE





# **A test of the ‘dear enemy’ effect in the winter wren: the roles of previous experience and song-type repertoire**

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Submitted

## ABSTRACT

The ‘dear-enemy’ effect, where neighbouring territory-holders display less aggression to one another than to strangers, is widespread in animals. This effect has been particularly well studied in male songbirds through vocal recognition of individuals, where it appears to be almost universal. However, at least two important aspects of such relationships remain poorly studied. First, the role of a territory-holder’s previous experience with particular intruders in developing and maintaining such relationships. Second, the extent to which such relationships are subject to proximate constraints on recognition, such as from song-type repertoire usage. We simultaneously tested these effects experimentally in the winter wren (*Troglodytes troglodytes*) with consecutive series of playbacks of both neighbour and stranger songs (adjacent to the boundary shared with the neighbour) with both small and large song-type repertoires. For each trial, we measured the latency of territory-holders to respond to the intrusion and the rate of singing in response. Our results showed that wrens display vocally-mediated dear-enemy relationships between neighbouring males, but that even a single intrusion experience with a previously unknown intruder can result in a substantial decrease in territorial response (longer latency to respond) to that individual. Furthermore, both the dear-enemy relationship between neighbours and the effect of previous experience were unaffected by the song-types used. We discuss the roles of previous experience with individual intruders and song-types in mediating territorial interactions among male birds.

## INTRODUCTION

A special case of territorial conflict and cooperation often occurs in territorial systems where displays by neighbours represent a lesser threat than non-neighbours or strangers, due to established territorial relationships existing between neighbouring individuals (reviewed in Ydenberg *et al.* 1988; Temeles, 1994). Neighbouring territory owners may then avoid costly and unnecessary confrontations by recognising and responding less aggressively to one another. Neighbour-stranger discrimination (NSD) of this type is referred to as the ‘dear-enemy’ effect (Fisher 1954; reviews in Ydenberg *et al.* 1988; Temeles 1994), and has been documented in numerous taxa, including mammals, birds, reptiles, amphibians, fish and insects (e.g. Barash 1974; Jaeger 1981; Pfennig & Reeve 1989; Armstrong 1991; Qualls & Jaeger 1991; Leiser & Itzkowitz 1999; Bee & Gerhardt 2001; Bee 2003; Leiser 2003; Husak 2004). Recognition through song in birds has been the most studied model, and results from experiments using song playback to territorial male songbirds indicate that vocally mediated NSD and the dear-enemy effect are almost ubiquitous in this group (reviews in Ydenberg *et al.* 1988; Temeles 1994; Stoddard 1996; see also Lovell & Lein 2004).

Although common and widespread, the expression of dear-enemy relationships could be strongly influenced by (a) the costs of reduced territory defence, and (b) proximate constraints on recognition. Between neighbouring songbirds, costs of reduced defence are most likely to arise from territory expansion or cuckoldry by neighbours (Gibbs *et al.* 1990; Hyman 2002). Benefits may also arise from reduced predation risk or increased foraging time. This may mean that dear-enemy relationships are commonly based on ‘tit-for-tat’ reciprocal (conditional) cooperation between neighbours, rather than by-product mutualism (Getty 1987; Godard 1993a; Hyman 2002; Olendorf *et al.* 2004). Consequently, one might expect that previous experience with neighbours would be of great importance, with territory-holders retaliating against ‘unreliable’ neighbours who trespass. Additionally, the costs of reduced defense could be related to the magnitude of the threat posed by a particular intruder, such as his ability to consolidate new territorial areas or successfully cuckold the territory-holder (Goddard 1993b; Olendorf *et al.* 2004). In principle, these threats may vary independently of the territory holder’s familiarity with the intruder. Therefore, signals of strongly aggressive intentions or sexual attractiveness, such as large or complex repertoires in birds (e.g. D’Agincourt & Falls 1983; Kramer *et al.* 1985; Catchpole & Slater 1995; Searcy & Yasukawa 1996; but see Peake *et al.* 2005), can also be expected to influence territorial aggression and the expression of the dear-enemy effect (Lambrechts 1992; Olendorf *et al.* 2004). Currently, little is known about the effects of previous experience on vocal recognition in birds, and species displaying the dear enemy effect offer a useful model in which to study this.

Proximate constraints on the ability of territorial individuals to signal their identity or reliably recognise each other might also influence dear-enemy relationships (Colgan 1983; Sherman *et al.* 1997). Although the mechanisms allowing NSD in songbirds remain largely unclear, previous research suggests that birds do not simply habituate to frequently heard songs or associate particular songs with particular locations (e.g. territory bound-

aries), but rather associate particular songs with particular individuals (e.g. Godard 1993b; Stoddard 1996; Langmore 1997). In order to do this, birds may use unique voice characteristics or minor variations in song-type versions between individuals, or they may learn all of the song-types in a frequently encountered individual's repertoire (e.g. Beecher *et al.* 1994). Constraints on recognition may then arise from song complexity or the number of different song-types used by an individual. Large repertoires may facilitate individual recognition by being more likely to contain song-types, combinations of songs or sequences of songs unique to an individual (Hultsch & Todt 1981). Birds may need to hear several different song-types to enable accurate discrimination (Weary *et al.* 1987). On the other hand, several authors have suggested that complex songs or large repertoires may hinder individual recognition, due to cognitive or temporal constraints on perceiving and remembering the differences between a large number of song variants and assigning particular patterns to particular individuals (reviews in Falls 1982; Stoddard 1996). Although many studies have provided good evidence for accurate NSD in many species, the effects of different song-types on recognition remain unclear (Naguib & Todt 1998).

In this study, we use simulated intrusions (song playbacks) to investigate whether territorial male Winter wrens (*Troglodytes troglodytes*) discriminate behaviourally between the songs of neighbours and strangers, and to what extent this effect is influenced by song-type repertoire and previous experience with intruders. Specifically, we aimed to address three questions. First, do wrens discriminate between intruding neighbours and strangers, and establish dear-enemy relationships, based on song? Male wrens vigorously defend stable long-term territories containing food and breeding resources, often year-round (e.g. Armstrong 1955; Cramp 1988). Acoustic signalling plays a central role in their intraspecific communication. Territorial intrusions among males occur frequently (Armstrong 1955; pers obs). These features make wrens a good candidate for displaying dear-enemy relationships (Temeles 1994). Second, does recent experience with individual intruders influence the subsequent response of male wrens to those intruders? In our experimental design, focal birds received two simulated intrusions by each of two intruders (a neighbour and a stranger). We compared the responses of focal males to intruders both before and after interacting with the same intruder in the preceding days. We predicted that if territorial relationships are based on reciprocal cooperation between individuals, males would show an elevated response to 'unreliable' individuals that had recently intruded onto their territory, particularly neighbours (Olendorf *et al.* 2004). Third, does the song-type repertoire of intruders affect individual discrimination? To test this, we compared playbacks featuring either two or five different song-types, and looked at the interaction between the song-types presented and whether an intruder was a neighbour or a stranger. Wrens sing complex songs in repertoires consisting of up to seven song-types, which are repeated in bouts of several songs before switching (Kreutzer 1974; Catchpole & Rowell 1993; own unpubl. data). These song-types show strong microgeographical variation (Catchpole & Rowell 1993).



## METHODS

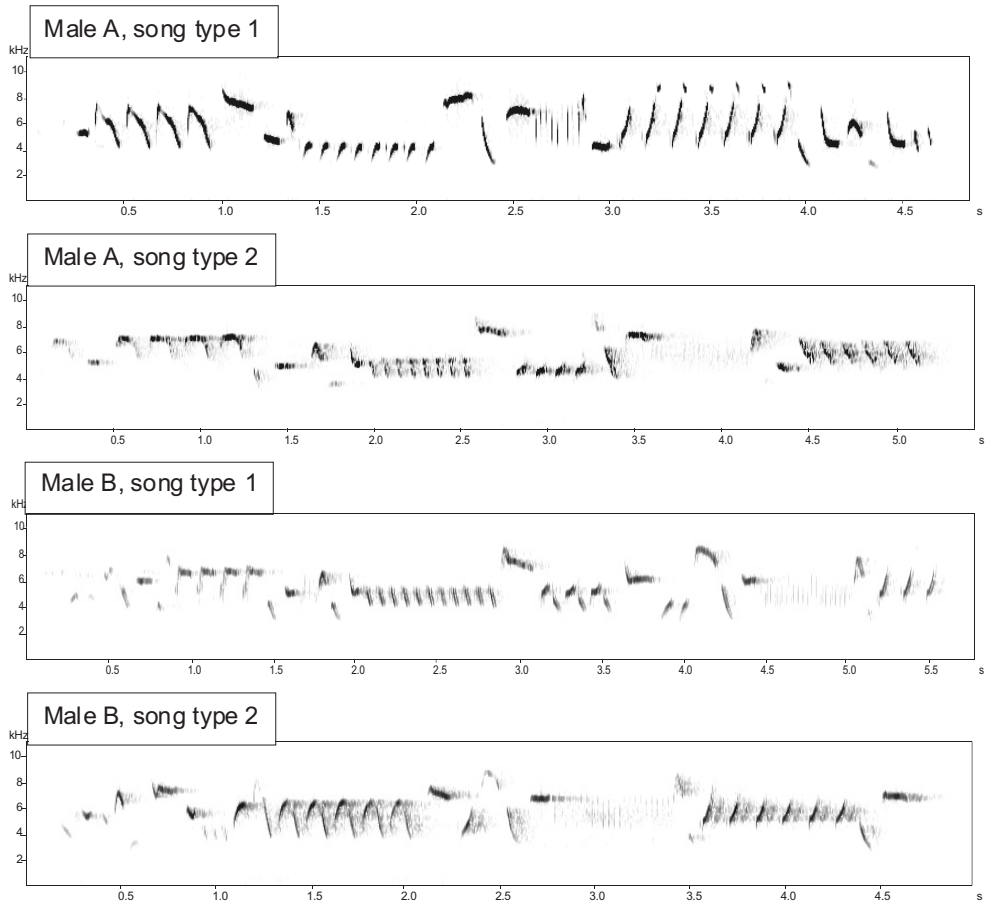
### Study area and population

This study was conducted in Vosbergen forest reserve, Paterswolde, The Netherlands (*ca.* 50 ha, 53°08' N, 6°35' E). Data were gathered during the main breeding season of the winter wren, in 2002. Adult male wrens were caught in mist-nests and colour-banded for individual identification. Sexes were distinguished based on the presence of a brood patch and behavioural cues such as singing and intra-sexual aggression (see Armstrong 1955). Territory borders were estimated by plotting the location of singing males and noting territorial interactions on a weekly basis. All individuals used in this study were breeding territorial residents.

### Playback experiments

In order to quantify intra-sexual territorial aggressiveness of male wrens, intrusions were simulated using a single speaker song playback of male wren song. Playback trials ( $n = 62$  trials on 16 focal males) were conducted from 10 June through 21 June 2002, between 0700 and 1000 h (the peak singing time of wrens at this time of the year (Armstrong 1955; chapter 4)). Territories are well established and vigorously maintained by male wrens at this stage of the breeding season. To create the playbacks, we recorded singing males in early June onto Sony Metal-XR cassettes using a Sony WM-D6C professional cassette recorder and a Telinga directional microphone with a fibreglass parabolic reflector. Each male was recorded for 20 minutes each day (*ca.* 50–60 songs) on two separate days between 0700 and 1000 h; as such each recording can be expected to contain the full repertoire of songs types used by each male recorded (Catchpole & Rowell 1993). Recordings were always made from *ca.* 30 m to reduce variation in sound degradation while not disturbing the singing male (this distance was sufficient to avoid alarm calling or interrupted songs caused by the presence of the recordist). Songs were digitized using a Sound Blaster Pro 16 sound card (Creative Technologies Ltd, Singapore) at 44.1 kHz and analyzed using SASLab Pro software (Avisoft Bioacoustics, Germany). Audio spectrograms were inspected visually and songs were classified into song-types based on the presence of a unique combination of syllables (see Catchpole & Rowell, 1993; Figure 1). Individual songs were filtered below 2 kHz to remove excess noise and transferred to compact discs (CDs) at a rate of four songs per minute. Four playback CDs were created for each focal male as follows: 1) a random selection of two song-types from a neighbour (a presumably familiar male with a directly adjoining territory) with each song-type played for one minute at a time and repeated five times ('small repertoire neighbour' trial), 2) a random selection of five song-types from the same neighbour with each song-type played for one minute at a time and repeated twice ('large repertoire neighbour' trial), 3) a random selection of two song-types from a presumably unfamiliar individual ( $\geq 4$  territories away) with each song-type played for one minute at a time and repeated five times ('small repertoire stranger' trial), and 4) a random selection of five song-types from the same unfamiliar individual with each song-type played for one minute at a time and repeated twice ('large repertoire stranger' trial). Thus, the playbacks differed in the





**Figure 3.1.** Audio spectrograms of two advertisement songs from two individual winter wrens, indicating variation in song-types within and between individuals.

number of song-types featured and whether the singer was a neighbour of a stranger to the focal bird. Additionally, the song-types used for the matched large repertoire and small repertoire playbacks were chosen at random from each recorded individual's repertoire. This approach was intended to reduce the possibility of habituation to particular song-types across the small and large repertoire trials performed on each focal male. In this way, only three of the 31 large repertoire playbacks consisted of the same combination of songs in the first two minutes as the corresponding small repertoire playback recorded from the same individual. The song rate and the rate of song-type switching were the same for all playbacks and within the natural range for wrens (Clark 1949; Armstrong 1944, 1955; Brémond & Aubin 1992; Catchpole & Rowell 1993; chapter 4). To avoid effects associated with a particular playback (Kroodsma 1989), different individuals were used as strangers for each focal male.

Playback trials were made using a Sony CFD-V3 portable CD player (Sony Corporation, Japan) adjusted to a natural volume of 67 dB at 10 m in front of the speaker. This approximately corresponds to the volume of naturally singing wrens (Holland *et al.* 2000). All focal males were separated by at least one territory, to reduce the chance of territory-holders reactions to playbacks being influenced by their eavesdropping on a previous playback in a neighbouring territory (Peake *et al.* 2001; Naguib *et al.* 2004). All playbacks (neighbour and stranger) for each focal male were performed from the same position, on the ground *ca.* 10 m inside the boundary between the focal male and the neighbour concerned, with the speaker facing the centre of the focal bird's territory. Thus, we tested for discrimination during a small-scale intrusion between stranger song and neighbour song at the correct boundary, and we avoided effects associated with different positions or previous interactions on other territorial boundaries (e.g. Langmore 1997). We presented playbacks in a crossover design with the neighbour-stranger and large repertoire-small repertoire treatments alternating and each male receiving each of the four possible treatments sequentially, such that there was an equal number of males receiving the neighbour treatment and large repertoire treatment first (Table 3.1). This design should eliminate any bias resulting from carryover effects of previous treatments. The four playback treatments (large repertoire neighbour song, small repertoire neighbour song, large repertoire stranger song and small repertoire stranger song) were performed one per day in a randomized order on each focal male, with the exception of one male which did not receive a large repertoire neighbour trial and one male which did not receive a small repertoire neighbour trial (due to the absence of the male from the territory or adverse weather conditions); thus we performed 62 playback trials on 16 focal males. To minimize seasonal effects, we performed all trials for each male on consecutive days with the exception of days with rain or high wind, in case this affected the response or sound transmission. This led to a mean interval between trials on each focal male of  $1.6 \pm 0.2$  days (range 1–8). All four trials for each male were conducted at the same time of day. We used two different observers who were randomly assigned to focal males, so that the same observer performed all four trials on each focal male to control for differences between observers. Each trial lasted for 10 min to allow enough time for the effects of a large repertoire to be assessed by the focal male. Before trials started, we confirmed that the focal male was present (observed singing) on his territory, to prevent large-scale variation associated with male presence or absence on the territory.

During each 10 min playback trial the following two response variables of the focal male were recorded by an observer hiding outside the territory of the focal male, 20m behind the speaker: 1) latency to approach within 20 m of the speaker, and 2) the number of songs sung within 20 m of the speaker. Simulated intrusions elicit a high level of aggression in wrens (see also chapter 4), and all focal males responded (within 20m) within the first five minutes of each trial (see Results). We interpreted shorter latencies and higher song rates as an indication of increased territorial aggression or motivation. These variables provide a useful indication of overall male territorial behaviour in wrens (chapter 4), which primarily consists of patrolling and searching for intruders, followed by protracted song contests between competitors. This approach also reduces problems

**Table 3.1.** The four treatment sequences forming the crossover experimental design used in this study. The use of neighbour or stranger playbacks was always alternated to each focal male. Large repertoire playbacks consisted of five song types and small repertoire trials consisted of two song types. Trials 1 to 4 indicate the order of trials. Focal males ( $n = 16$ ) were randomly assigned to one of the four experimental sequences. \* indicates experimental combinations performed on three focal males; all other combinations performed on four.

	Sequence 1	Sequence 2	Sequence 3	Sequence 4
First trial	Neighbour Large repertoire	Stranger Large repertoire	Neighbour Small repertoire*	Stranger Small repertoire
Second trial	Stranger Small repertoire	Neighbour Small repertoire	Stranger Large repertoire	Neighbour Large repertoire
Third trial	Neighbour Small repertoire	Stranger Small repertoire	Neighbour Large repertoire	Stranger Large repertoire
Fourth trial	Stranger Large repertoire	Neighbour Large repertoire*	Stranger Small repertoire	Neighbour Small repertoire

with conflicting or meaningless measures (Stoddard 1996). We also recorded closest approach distance in four distance classes (0-5 m, 5.1-10 , 10.1-20 m, >20 m), but we did not analyze this variable due to lack of variation between trials; during most trials (76%) males approached <5 m to the speaker (93% <10m). We did not record precise distances to the playback due to the difficulties of assessing this during experiments, and because this depends heavily on the distribution and layout of the vegetation surrounding the speaker. All conclusions reported regarding latency were qualitatively the same when considering latency <5 m. Where recorded separately, song rates during the first 5 min of each playback with correlated significantly with song rates during the second 5 min ( $r_s = 0.348$ ,  $n = 56$ ,  $P = 0.009$ ). We never observed alarm calls during trials, and neighbouring individuals or females were not observed to interfere with the experiments (never approaching within 20 m).

### Analyses of playback experiments

Statistical analyses were performed in SPSS 12.0.1 for Windows (SPSS Inc., USA) and followed Quinn & Keough (2002). As is common with such data, latency displayed a log-normal distribution (skewness  $1.582 \pm 0.304$  SE) and was therefore log<sub>10</sub>-transformed prior to all analyses to improve normality and homoscedascity (skewness following transformation  $0.263 \pm 0.304$  SE). To model within-subjects effects in our repeated measures design, all analyses were performed using the linear mixed model (MIXED) procedure with restricted maximum likelihood (REML) estimation, and included male identity as a 'subject' (random) effect and trial number (1-4) as a 'repeated' effect. We set the repeated covariance type to scaled identity (assumes constant variance and zero covariance), which provided qualitatively similar estimates to unstructured and first-order autoregressive covariance types and provided the best fitting model as assessed by information criteria

and changes in deviance. Unless stated otherwise, we reduced models by excluding non-significant (fixed effect  $P > 0.1$ ) higher-order terms from the saturated model; all main effects of interest were retained in each model. To present  $\log_{10}$ -transformed latency data (Figure 2 and 3), we show the geometric mean ( $10^{\text{mean}} \pm \text{standard error (SEGM, } 10^{\text{mean} \pm \text{SE}}$ ); the geometric mean (which approximates the median, but is lower than the arithmetic mean) is less affected by extreme values than the arithmetic mean and produces an asymmetrical SE. Similarly, for model-derived estimates of effect sizes of  $\log_{10}$ -transformed latency data we report back-transformed estimates of means ( $10^{\text{estimate}}$ ), which represent the ratios of the geometric mean of latency between the treatments (i.e. an estimate of 1 indicates no effect; Sokal and Rohlf 1995). For song rate, estimates represent songs per minute. Estimates of fixed effects are reported with Type III tests of significance (t-statistics and  $P$ -values), along with 95% confidence intervals (CI) to indicate accuracy and aid the interpretation of non-significant results. All  $P$ -values are two-tailed and results are considered significant when  $P < 0.05$  unless otherwise stated.

## RESULTS

The results of the model considering the effects of whether the intruder was a neighbour or stranger, the number of song-types used (two or five) and previous experience of the same intruder are summarized in Table 3.2. The mean latency to respond during all trials was  $65 \text{ s} \pm 8.8 \text{ SEM}$  (range 9-290 s). We found that latency was significantly longer in response to large repertoire playbacks than to small repertoire playbacks (Figure 3.2). In addition, latency was significantly shorter when the focal male had no previous experimental experience of the intruder compared to when he did, but there was also a significant interaction between whether the playback was from a neighbour or a stranger, and previous experimental experience of the intruder. Latency was longer in response to stranger playbacks that had been previously encountered compared to strangers that had not been encountered (Figure 3.3). Overall there was no significant effect of whether a playback was from a neighbour or stranger when controlling for previous experience and the interaction (Table 3.2). However, when considering only trials where the focal male had no previous experimental experience of the intruder (i.e. first and second trials, Table 3.1) latency was significantly longer to neighbour playbacks than stranger playbacks (estimate 2.487 (95% CI 1.385-4.466),  $t_{13.315} = 3.353$ ;  $P = 0.005$ ; song-type repertoire: estimate 1.414 (95% CI 0.781-2.560),  $t_{14.146} = 1.251$ ,  $P = 0.231$ ; Figure 3.3).

In contrast to the results with latency, we did not detect any significant main effects on song rate of any of our experimental treatments (Table 3.2). The mean song rate during all trials was  $4.0 \pm 0.2 \text{ SE}$ . When included in the final reduced model (as presented in Table 2), there were no significant interactions between whether the playback was of a neighbour or stranger, and song-type repertoire (latency: estimate = 0.828 (95% CI 0.356-1.926),  $t_{40.489} = 0.451$ ,  $P = 0.654$ ; song rate: estimate = 0.347 (0.024-4.941),  $t_{41.192} = 0.805$ ,  $P = 0.425$ ), or between playback song-type repertoire and previous experimental experience with the intruder (latency: estimate = 0.810 (0.342-1.920),

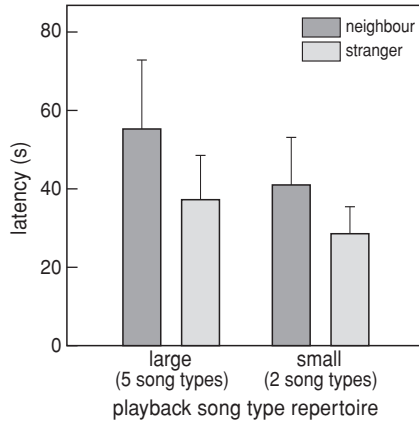
**Table 3.2.** Estimates of fixed effects (with 95% confidence intervals and statistics) of response variables (log<sub>10</sub> latency to approach <20m, song rate) during each trial for the four playback treatments derived from repeated measures model. Fixed effects included playback identity (neighbour-stranger), number of song-types used in the playback (repertoire) and previous experimental experience of the same intruder (experience); non-significant ( $P > 0.1$ ) higher order terms were excluded from the saturated model and the reduced model is reported (see text). Estimates for the log<sub>10</sub>-transformed latency data have been back-transformed, and thus represent the ratios of the geometric mean of latency (approximates to the median) between the treatments (i.e. an estimate of 1 indicates no effect). Estimates for song rate represent songs per minute. Significant effects are in bold.  $N = 62$  playback trials on 16 individual males (see Table 3.1).

Response variable	Estimate (95% CI)	Comparison†	<i>t</i>	df	<i>P</i>
Latency (s)	0.855(0.481-1.522)	Neighbour-stranger	0.547	42.493	0.587
	<b>1.577(1.038-2.395)</b>	<b>Repertoire</b>	<b>2.199</b>	<b>42.492</b>	<b>0.033</b>
	<b>0.494(0.278-0.877)</b>	<b>Experience</b>	<b>2.479</b>	<b>42.301</b>	<b>0.017</b>
	<b>2.986(1.300-6.860)</b>	<b>Neighbour-stranger x Experience</b>	<b>2.653</b>	<b>42.449</b>	<b>0.011</b>
Song rate (songs/min)	-0.039(-0.590-0.512)	Neighbour-stranger	0.143	42.281	0.887
	0.155(-0.398-0.709)	Repertoire	0.565	42.369	0.575
	-0.108(-0.661-0.446)	Experience	0.392	42.369	0.697

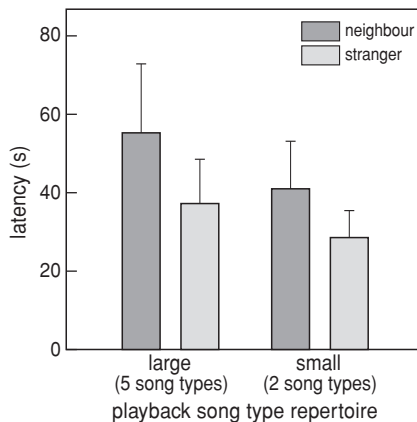
†Neighbour-stranger: playback of a neighbour compared to playback of a stranger; Repertoire: playback of a large repertoire (5 song types) compared to playback of a small repertoire (2 song types); Experience: no previous experimental experience of the intruder compared to previous experience; Neighbour-stranger x Experience: interaction

$t_{43.031} = 0.493$ ,  $P = 0.625$ ; song rate: estimate = 0.485 (0.031-7.548),  $t_{42.573} = 0.0532$ ,  $P = 0.598$ ). Similarly, when considering song rate there was no significant interaction between whether the playback was of a neighbour or stranger and previous experience (estimate = 0.157 (0.011-2.152),  $t_{41.220} = 1.428$ ,  $P = 0.161$ ).

The latency time during trials when the focal bird had previous experimental experience with both intruders (neighbour and stranger) was estimated to be 2.024 (95% CI 1.140-3.997) times as long as when the focal bird had no previous experimental experience of either intruder ( $P = 0.017$ ; Table 3.2). Although it is possible that such a decline in territorial response or vigilance with previous experience of an intruder could arise due to habituation to the experimental procedure, we expected to be able to distinguish this from previous experience of each individual intruder because our experimental design alternated neighbour and stranger trials (Table 3.1). Accordingly, the interaction between previous experience and whether the playback was of a neighbour or stranger ( $P = 0.011$ ; Figure 3), along with the relatively good fit of the simple scaled identity repeated covariance structure, indicate that habituation to the experimental intrusions was not an important determinant of the territorial response. The scaled identity structure assumes constant fit and no correlation between any elements (see Methods). Furthermore, when tested as a fixed effect (controlling for within-subject random effects), trial number (1-4)



**Figure 3.2.** Latency (s) to respond to playback (approach within 20m) plotted against playback song-type repertoire (two or five song-types). Latency was significantly longer to large repertoire playbacks (five song-types) than small repertoire playbacks (two song-types;  $P = 0.033$ ). Grey bars represent neighbour playback, white bars represent stranger playback. Values shown are the geometric mean  $\pm$  SEGM (which is smaller than the arithmetic mean). Males ( $n = 16$ ) are each represented four times (once for each level of neighbour or stranger playback  $\times$  song-type repertoire), except for two males represented only three times (see Table 3.1).



**Figure 3.3.** Latency (s) to respond to playback (approach within 20m) plotted against previous experimental experience with the same intruder. Grey bars represent neighbour song playback, white bars represent stranger playback. There was a significant effect of previous experience ( $P = 0.017$ ) and a significant interaction between previous experience and whether the playback was of a neighbour or a stranger ( $P = 0.011$ ). When comparing only trials where the focal male had no experience with the intruder, latency was significantly longer to neighbours than to strangers ( $P = 0.005$ ). Values shown are the geometric mean  $\pm$  SEGM (which is smaller than the arithmetic mean). Males ( $n = 16$ ) are each represented four times (once for each level of neighbour or stranger playback  $\times$  previous experience; trials performed 1-8 days apart), except for two males represented only three times (see Table 3.1).

did not have a significant effect on latency (estimate = 1.062 (0.870-1.298),  $t_{45.937} = 0.609$ ,  $P = 0.545$ ) and explained only 1.69% of variation in latency. This further indicates that a simple habituation effect was not responsible for the association between previous experience with an intruder and latency.

## DISCUSSION

### The “dear-enemy” effect and previous experience

We used song playback to determine whether male wrens differ in their territorial response in relation to the whether an intruder was a neighbour or a stranger, the song-type repertoire sung by the intruder, or their previous recent experience with an intruder. When considering latency to approach only during the first trials (i.e. those when the focal male had no previous experimental experience with the intruder), we did detect a strong dear-enemy effect in wrens as expected in the form of shorter latencies to respond to strangers compared to neighbours (Figure 3.3). Thus, our results provide evidence that wrens are able to discriminate between neighbouring and stranger conspecifics based on song, and respond accordingly in a territorial context (i.e. the dear-enemy effect).

However, our results indicated that the dear-enemy effect is strongly mediated by previous experience with non-neighbouring intruders. Latency to approach the speaker was shorter for stranger than neighbour intruders when the focal male had no previous experience with each intruder, but was similar when he had recent experimental experience with the same intruders. This result is surprising, as we predicted that males would increase their response towards individuals that had recently intruded onto their territories because such individuals would be deemed untrustworthy, and that this shift would be stronger for intruding neighbours if dear-enemy relationships were based on reciprocal cooperation (Hyman 2002; Olendorf et al 2004). In fact, this pattern was absent for neighbour playbacks. Wrens evidently did not retaliate against untrustworthy neighbours. For stranger playbacks, this pattern went in the opposite direction (after only one recent intrusion experience), i.e. wrens responded less quickly to previously unknown intruders with whom they had recent experience. This result is in line with Hyman’s (2002) study on Carolina wrens (*Thryothorus ludovicianus*), although Godard (1993b) found that male hooded warblers (*Wilsonia citrina*) responded more aggressively to neighbours after experiencing an intrusion by the same neighbour compared to a stranger earlier the same day. Similarly, Olendorf *et al.* (2004) found that red-winged blackbirds (*Agelaius phoeniceus*) displayed increased vigilance against intruding neighbours that persisted for days. In contrast, our results suggest that the relationships between neighbouring winter wrens are quite stable and not greatly influenced by small-scale incursions into each other’s territories. Furthermore, in contrast to the surprising results of Hyman (2002), our data support the idea that intrusions by strangers should not affect dear-enemy relationships between neighbours, because we did not find that latency to approach neighbour playbacks was changed following experimental intrusions. One potentially important difference between our study and that of both Godard (1993b) and Hyman (2002) is that our trials were per-



formed on different days rather than in a single day, which may have given neighbouring birds a chance to re-establish dear enemy relationships following experimental intrusions. Nevertheless, it is unclear why wrens responded less strongly to previously unknown intruders with whom they had recent experience (after only one intrusion experience), but intruder-specific habituation may be the most likely mechanism (Bee 2003). Wrens that respond less quickly to known intruders that have been successfully overcome in previous contests, may benefit by decreasing the likelihood of escalated contests when the outcome should be predictable. Alternatively, it may only take one interaction with a previously unfamiliar male for him to be considered a trustworthy neighbour, but this seems unlikely to explain our results given the number of repeated interactions between wrens and their neighbours throughout each day. To our knowledge, no previous studies have considered territorial responses to previously unknown intruders (non-neighbours) following a recent intrusion.

In contrast to the results with latency, we found no significant effects of any experimental treatment (whether the playback was of a neighbour or stranger, song-type repertoire or previous experimental experience with intruders) on song rate, with estimated effect sizes of  $<0.2$  songs per min (Table 3.2). One explanation for this discrepancy is that, after detecting and approaching the intruder, focal males spent time on aggressive behaviour not measured here other than attempting to sing more songs relative to their opponent. Such behaviour might include antiphonal singing (Brémond & Aubin 1992), elongating songs or song-type matching (e.g. Krebs *et al.* 1981; McGregor & Horn 1992; Dabelsteen *et al.* 1996; Balsby & Dabelsteen 2001; Burt *et al.* 2001; M. Berg unpubl. results), searching for the intruder or performing visual displays (Armstrong 1955; Catchpole 1989; Peake *et al.* 2005). Alternatively, approach behaviour and song rate during singing contests may represent different kinds of information; in wrens, song rate during singing contests may function primarily in quality advertisement rather than as a direct form of territory defence (chapter 4).

### **Territorial interactions and song-type repertoires**

There are several ways in which song-type repertoire has been suggested to influence territorial responses: larger repertoires may indicate sexual attractiveness (e.g. Catchpole & Slater 1995; Hasselquist *et al.* 1996) or aggressive intentions (e.g. D'Agincourt & Falls 1983; Kramer *et al.* 1985), or repertoires of song-types may operate on individual recognition (e.g. Falls 1982; Weary *et al.* 1987; Stoddard 1996). Although song clearly plays a major role in the territorial behaviour of many bird species including wrens (e.g. Catchpole & Rowell 1993; Catchpole & Slater 1995; Stoddard 1996), relatively little is known about the role of song-type repertoires in territory defence or individual recognition.

Our results indicate that wrens responded with shorter latencies to the small repertoire playbacks (two song-types repeated in five bouts) compared to the large repertoire playbacks (five song-types repeated in two bouts). This effect could potentially be related to the size of the song-type repertoire, song-type usage (i.e. continual switching to novel song-types as opposed to repetition of previously used song types), or the actual song-

types used. While the song rate and rate of song-type switching was constant across all playbacks, in most cases (90%) the song-types used were different, with the matched small repertoire and large repertoire playbacks produced from each recorded male presenting a different combination of song-types in the first two minutes in 28 out of 31 cases (90%). In our study the differences in song-type repertoire size and song-type repetition (i.e. repeating previously sung song-types in a single singing bout) between the large and small repertoire playbacks were only revealed after the first 2 min. During the first two minutes of playback, both small and large repertoire playbacks presented two different song-types, each repeated for 1 min. However, the mean latency time in our trials was only  $65 \text{ s} \pm 8.8 \text{ SE}$ , and in 48 (77%) of the trials the focal male responded in less than 2 min. Furthermore, of the 14 trials that elicited a latency to approach the playback of  $>2$  min, only nine (64%) were large repertoire trials. Thus, most males in our study could not have been influenced by differences between small and large repertoire playbacks in the number of song-types presented or song-type usage. Instead, it seems likely that this effect arose at least in part from unknown differences in the song-types themselves presented by small and large repertoire playbacks in the first two minutes of the playback. Although the importance of song repertoires and song structure on female mate choice has been well established, far fewer studies have addressed the question of how variation in song-types and song structure can influence territorial interactions (e.g. Catchpole & Slater 1995; Leitão & Riebel 2003; Collins 2005; Illes *et al.* 2006; Leitão *et al.* 2006). Further study will be needed to unravel the role of song-type variation in territorial interactions in winter wrens.

A further question of this study was whether hearing different song-types might facilitate (Weary *et al.* 1987; Stoddard 1996) or hinder (Wiley & Wiley 1977; Krebs & Kroodsma 1980; Falls & d'Agincourt 1981; Searcy *et al.* 1981; Falls 1982) individual discrimination by focal males. We found no significant interactions between the song-types presented on the playback and either the focal male's previous experience with the intruder or whether the playback was of a neighbour or stranger (Fig. 1, Table 2). To date only a few studies have tested directly the hypothesis that song-type repertoire influences neighbour-stranger discrimination within the same or closely related species, although comparative analyses have suggested that species that possess larger repertoires exhibit weaker neighbour-stranger discrimination. Weary *et al.* (1992) have shown that both American redstarts (*Setophaga ruticilla*) and yellow warblers (*Dendroica petechia*) discriminated equally well between neighbours and strangers when presented with playbacks of a repertoire of song-types in series ('serial mode') or a single song-type repeatedly ('repeat mode'). Furthermore, the yellow warbler showed discrimination as strong as the redstart despite having a substantially larger repertoire. Similarly, Godard & Wiley (1995) found that male hooded warblers discriminate neighbour songs broadcast from the correct shared boundary from those broadcast from the opposite side of the focal male's territory regardless of whether playbacks of one song-type ('repeat mode') or two song-types ('mixed mode') were used. In most cases in these studies the song-types used for repeat mode singing differed from those used for serial or mixed mode. This means that there is a possibility that song-types used in a particular singing mode have features that promote neighbour-

stranger recognition other than the differences in the number of song-types used. In contrast, wrens have only a single mode of singing, and the song-types that we used for each matching pair of large repertoire and small repertoire playbacks were chosen at random from the same repertoires.

### Conclusions

Overall, our results indicate that wrens, like most songbirds (reviews in Ydenberg *et al.* 1988; Temeles 1994; Stoddard 1996), are able to discriminate between individuals based on song and display a stronger response (shorter latency to respond) to intruding strangers which have not been encountered previously than to familiar neighbours. However, this was only true when the territorial male had never encountered the stranger before. This discrimination ability did not seem to be affected by variation in song-type repertoire within the natural range, although song-type repertoire did have a surprising effect on territorial response overall. Thus, our results provide no indication of either a cost or benefit to song repertoires in terms of individual recognition. Further, our results suggest that dear-enemy relationships in wrens are robust to at least a low-level of intrusion between neighbours. However, it appears that territorial relationships between non-neighbouring wrens are highly flexible and adjusted rapidly to experience with previously unknown individuals. The dynamics of this relationship, together with the role of song-type repertoires in male-male interactions, deserve further attention in future studies.

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