REVIEW

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Quasi-parasitism in birds

Abstract Our view of avian mating systems has been revolutionised by the use of molecular tools that have provided evidence supporting theoretical predictions that extrapair paternity (EPP) and intra-specific brood parasitism (IBP) would be widely observed alternative mating strategies in socially monogamous species. Quasi-parasitism (QP) is a third type of alternative mating strategy, where a female lays an egg in another female's nest and that egg is fertilised by the male partner at the parasitised nest. In contrast to both EPP and IBP, QP has been reported in only 12 species to date. We explore reasons for the apparent rarity of QP in birds and conclude that it is only likely to be adaptive in a fairly restrictive set of circumstances. We also review all of the evidence for the occurrence of QP in birds and find that it is far more limited than generally believed, as many apparent examples may be explained by rapid mate–switching or errors in molecular analysis of parentage. We suggest a number of criteria that need to be met for an unequivocal demonstration that QP has actually occurred.

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Introduction

"These 'bastards' are the result of three different sources of parental uncertainty: Extra-pair copulation, intra-specific egg parasitism, and quasi–parasitism (parasitism by a female who was fertilized by the male attending the parasitized nest)" (Wrege and Emlen 1987).

Petrie (1986) was the first to speculate that, in some species, a host male might allow parasitic females to lay eggs in his nest in exchange for extrapair copulations. This idea emerged from Petrie's (1986) behavioural analysis of reproductive strategies in male and female moorhens (*Gallinula chloropus*). Wrege and Emlen (1987), however, were the first to use the term quasi–parasitism for this phenomenon (as quoted above), in their list of the various ways that white-fronted bee-eater (*Merops bullockoides*) parents might end up caring for chicks that were not their genetic offspring. Because theirs was one of the earliest studies to use molecular methods to investigate familial relationships in birds, each of these reproductive tactics was approached as an equally likely alternative that could be revealed by these new techniques. This, after all, was a time when behavioural ecologists were still coming to terms with the idea that 'monogamous' birds were capable of extrapair copulations (and extrapair paternity), and there was no reason to suppose that one source of parental uncertainty was more likely than another; all tactics seemed equally plausible and there was little theory available to guide research. Moreover, the molecular methods available to those earliest studies were generally not capable of identifying genetic parents with any certainty.

In the intervening 18 years, considerable attention has been given to the study of genetic parentage in birds, using increasingly refined molecular methods. Now, with a wealth of data on more than 130 bird species, we are in a position to conclude that the three alternative breeding

tactics, extrapair paternity (EPP), intraspecific brood parasitism (IBP), and quasi-parasitism (QP), are not equally likely. Overwhelmingly, extrapair parentage occurs in the form of EPP with 90% of the 130+ bird species studied so far having at least a small percentage of broods with offspring fathered by a male other than the female parent's social partner. Variation in the level of EPP among species is extreme and the incidence can exceed 70% of chicks in 95% of broods (reviewed in Griffith et al. 2002).

IBP is also relatively common in birds, occurring in 20 of the 69 species studied to date with molecular techniques (Arnold and Owens 2002). Most examples of IBP come from species, like the moorhen, in which females having a nest of their own appear to deliberately lay some of their eggs in the nests of conspecifics (Gibbons 1986), or from species where parasitism is used by non-breeding females as a conditional tactic to deal with nest site or territory saturation (Eadie and Fryxell 1992). In some other species, IBP occurs very rarely, at a frequency more suggestive of accidental dumping than an adaptive strategy (reviewed by Yom-Tov 1980). IBP seems to occur most often when individuals nest close together and breeding is synchronous (Rohwer and Freeman 1989). A recent comparative analysis also reveals a strong positive association between the frequency of IBP in a species and both its clutch size and its annual fecundity (Arnold and Owens 2002).

QP, on the other hand, is relatively rare, reported so far in only 12 species (Table 1). The spate of recent reports might suggest that this alternative mating tactic was missed in earlier studies. However, in this paper, we find little convincing evidence that QP is an adaptive behaviour in birds. For this analysis, we reviewed all of the literature on genetic parentage in birds to the end of 2003 to search for examples that might be construed as QP, whether or not the authors made such a claim. In addition, we show why QP is expected to be rare in birds and present a summary of the evidence that would be needed to conclusively demonstrate its existence. Unlike EPP and IBP, solid evidence for QP requires both careful behav-ioural observations and the genetic analysis of both paternity and maternity within a brood. In addition, evidence that QP is adaptive for one or both of the adult participants requires demographic data so that the costs and benefits of QP can be evaluated.

Molecular evidence for quasi-parasitism

Whereas IBP can be detected simply by the appearance of a supernumerary egg in a nest, QP can only be inferred when there is some molecular evidence to show that the attendant male at the nest has fertilised the 'parasitic' egg. Indeed, in most studies claiming evidence for QP (Table 1), parasitic eggs are identified only by genetic analysis, rather than direct observation of egg-laying by brood parasitic females or other means of distinguishing brood-parasitic eggs. Three molecular techniques for genetic analysis, allozymes, multilocus minisatellites, and microsatellites, have so far been employed to look for QP, and we catalogue the strengths and weaknesses of each technique here.

Electrophoretic analysis of weakly polymorphic protein allozymes was the first technique deemed suitable for paternity assessment in birds. Whilst revolutionary at the time, allozyme analysis was far from satisfactory for this purpose due to the low level of polymorphism at each locus, the few loci that were generally scored, and the fact that each allozyme revealed by this method could actually be determined by >1 true alleles at the DNA sequence level (Lewontin 1974). Thus, allozyme analysis could be used to reveal the minimum level of extrapair parentage in a brood, but some true genetic mismatches may have been missed. As a result, the probability of detecting mismatched fathers using allozymes was usually 0.40–0.70 (Burke 1989). Moreover, parentage can rarely be positively assigned using this method as offspring often match other adult males and females in the sample.

Many of these problems were solved with the advent of multilocus (minisatellite) DNA fingerprinting in 1985 (Jeffreys et al. 1985). Bands on autoradiographs produced

Table 1 Background information on published reports of quasi-parasitism in birds: the number of broods sampled for extrapair parentage, the number of years (breeding seasons) over which those broods were sampled, whether those broods were from colonial or solitary nests, and whether detailed observations were reported that might have detected evidence of extrapair copulations, IBP, or mate switching

Species	Location	Years	Broods	Nest dispersion	Observations?	Reference
White-fronted bee-eater	Kenya	3	65	Colonial	Yes	Wrege and Emlen 1987
Eastern kingbird	Michigan, USA	3	19	Solitary	No	McKitrick 1990
Zebra finch	Victoria, Australia	1	16	Colonial	Yes	Birkhead et al. 1990
Bearded tit	Austria	2	44	Both	No	Hoi and Hoi-Leitner 1997
Penduline tit	Slovakia	2	68	Solitary	No	Schleicher et al. 1997
Water pipit	Switzerland	3	258	Solitary	Yes	Reyer et al. 1997
Sand martin	Scotland	3	45	Colonial	Yes	Alves and Bryant 1998
Black-capped chickadee	Ontario, Canada	4	58	Solitary	Yes	Otter et al. 1998
Kentish plover	Turkey	3	65	Solitary	No	Blomqvist et al. 2002
Common sandpiper	Sweden	2	25	Solitary	No	Blomqvist et al. 2002
House sparrow	Austria	1	46	Colonial	No	Vaclav et al. 2003
Barn swallow	Spain	1	170	Colonial	No	Møller et al. 2003

by this method are codominant markers that segregate in a Mendelian fashion. Autoradiographs are scored using a variety of techniques to distinguish among bands and parentage is usually assessed by calculating band-sharing coefficients and the number of novel bands on a gel (Burke 1989). This method was widely used through the 1990s and seems to reveal quite accurately the average proportions of EPP in a sample, as probabilities of falsely identifying a nestling as extrapair are typically in the order of 10^{-2} to 10^{-8} (Burke 1989).

Multilocus fingerprinting has been less successfully used to identify actual extrapair parents with any degree of certainty. Usually, in studies of EPP using this technique, the pair male is assumed to be the father unless he is excluded but, when he is excluded, the true (extrapair) father remains unidentified. In studies claiming QP, however, this is a critical issue as the pair male must be reliably identified as the father of any extrapair offspring in his nest. With multilocus DNA fingerprinting, identification of true parents is problematic because minisatellite mutation rates are relatively high (Burke 1989), the sizing and matching of bands from different individuals is usually accomplished with an unknown error rate (Galbraith et al. 1991; Queller et al. 1993), and the number of bands scored is typically relatively small (<25) and highly variable across individuals within a single study. The difficulty of comparing bands across lanes within gels and between gels, and considerable variability in the number of bands scored per individual, means that the assignment of parentage for some offspring will be quite ambiguous.

Thus, the probabilities of identifying true parents that are realised in forensic work are rarely found in wildlife research due to the large number of multilocus DNA fingerprints that would be required to achieve such a level of certainty. Even the most successful wildlife studies that have identified extrapair males rarely locate more than half of them with any accuracy, despite some liberal assumptions (e.g. Westneat 1990). Since EPP is fairly common in birds, the rare false exclusion of a male (i.e. a false indication of EPP) would result in relatively small error in the calculation of EPP rates. QP, on the other hand, is exceedingly rare, so the occasional false indications of QP due to such ambiguity could account for many of the currently known examples.

Some researchers have also used multilocus DNA profiling to test whether a model of EPP, IBP or QP could best explain the distribution of non-kin in a population. This "probabilistic modelling approach" is based on assumptions derived from behavioural data (see Wrege and Emlen 1987, Westneat et al. 1987) and does not require that all instances of offspring resulting from each tactic be reliably identified. Unfortunately, this approach assumes that a single tactic accounts for all non-kin in the sample, and requires large numbers of non-kin to provide sufficient statistical power to construct and compare the models reliably (Wrege and Emlen 1987). To the best of our knowledge this approach has been successfully applied in only a single study (Westneat 1990), where all non-kin were identified as resulting from EPP.

The most recent, and in our opinion most reliable, method for assessing parentage in birds is microsatellite DNA profiling (Queller et al. 1993). With this method hetero- and homozygotes are revealed by two bands and one band, respectively, so that scoring of bands is usually less equivocal than with multilocus fingerprinting. With enough highly polymorphic primers (typically 5–10), the probability of identifying individual parents in a population can be quite high (>99%; Queller et al. 1993), such that both parents of putative QP offspring can be reliably identified if DNA is available from all potential candidates. At the very least, for a given brood, the attending male can be reliably identified as the father and the attending female can be reliably excluded as the mother, thus revealing either QP, polygyny, or mate switching (see below). In combination with the appropriate behavioural data, parentage assignment using microsatellites can therefore provide unequivocal evidence for QP.

Behavioural evidence for quasi-parasitism

Identifying QP solely on the basis of genetic evidence without any supporting behavioural or ecological information can be problematic, for two reasons. First, for any genetic method, errors in sizing DNA fragments (bands) can lead to the false rejection of a female as the true genetic parent, and thus the incorrect conclusion that QP has occurred. Second, genetic evidence consistent with QP can arise as a result of mate switching during egg-laying, where the replacement female lays in the same nest as her predecessor.

Unambiguous evidence of the occurrence of QP demands behavioural information in addition to molecular data. First, observational evidence for egg dumping should comply with the generally accepted criteria in the IBP literature (e.g. Yom-Tov 1980; Brown 1984; Gibbons 1986; Lyon 1993a). Abnormally large clutches, late-hatching eggs, or eggs that appear in the clutch at unexpected intervals, may lead to suspicion that an egg has been dumped. However, the most reliable evidence is the appearance of two or more new eggs on the same day (Brown 1984; Gibbons 1986; Lyon 1993a; McRae 1997) because individual female birds are physiologically incapable of laying more than one egg per day (Sturkie 1965). It is particularly unwise to base suspicion of egg-dumping simply on colour and pattern features of eggs in a clutch (see McRae 1997) and more confidence should be placed on observations combining unique features of a particular egg with an unexpected laying time (e.g. Lyon 1993a).

Second, as for all studies of paternity, it is important to identify all of the "within-pair" parents associated with a brood, either by catching adults during incubation or chick rearing, or by observing marked individuals exhibiting parental behaviour at the nest. Even in socially monogamous species, rare cases of polygyny and polygynandry can occur (e.g. Kempenaers 1993) wherein a second female socially associated with a single brood is not an extrapair female (hence her offspring will not be

the result of QP). Problems can also arise with rapid nest or territory takeovers, or rapid mate-switching providing examples of apparent EPP, IBP and QP, but these too can be resolved by careful observations to identify within-pair individuals through the breeding season.

Third, it is necessary to identify the sex of both putative parents. If the sexes are mixed up, then a straightforward case of extrapair paternity will be misinterpreted as a case of QP. Even in cases where it is easy to identify sex using morphological traits, it is a good idea to use a molecular sex marker (e.g. Griffiths et al. 1998) as an additional quality control.

QP as an adaptive reproductive strategy

Quasi-parasitism occurs at the intersection of two reproductive tactics, EPP and IBP, that are typically independent with respect to a given offspring. In populations where both of these tactics occur at high frequencies on a local spatial scale, some instances of QP may occur by chance, rather than resulting from behaviours specifically selected to promote cooperation between the host male and the parasitic female. Such 'chance QP' (Alves and Bryant 1998) will occur when a female lays a parasitic egg sired by an extrapair male (EPP) and that egg is, by chance, laid in the nest of the extrapair male (Fig. 1). Adaptive QP, in contrast, involves a clear causal link between an extrapair mating and subsequent brood parasitism that provides a fitness benefit to one (the female) or both participants.

To distinguish adaptive QP from chance QP, information is needed on the frequencies of EPP and IBP in a population, as well as on the spatial pattern of their occurrence (i.e. the number of territories or mated pairs that could provide potential host nests or EPP partners for a given female or male). With this information, the expected frequency of chance QP can be readily estimated for any population (Fig. 1). All else being equal, the fewer the territories over which either (1) females search for host nests or (2) both sexes pursue EPP, the greater the likelihood that EPP and IBP will co-occur by chance for a given egg (Figure 1). Thus, with moderate levels of both random EPP (e.g. 15% of eggs) and random IBP (e.g. 10% of eggs), and a small neighbourhood size (e.g., three nests) like that in some colonial species, QP would account for about 0.5% of eggs laid in the population, by chance alone (Fig. 1). There is little merit in the investigation of such chance QP since the independent assessment of EPP and IBP will tell the whole story. Whenever QP occurs at a significantly higher frequency than predicted by chance, or at a higher frequency than IBP (Alves and Bryant 1998), QP is likely to be adaptive for one or both participants. The next step is to examine the behavioural context and fitness consequences.

Since QP involves both a parasitic female and host male, the potential fitness benefits to each participant must be evaluated to determine how QP is adaptive. For example, compelling evidence that QP is adaptive for the
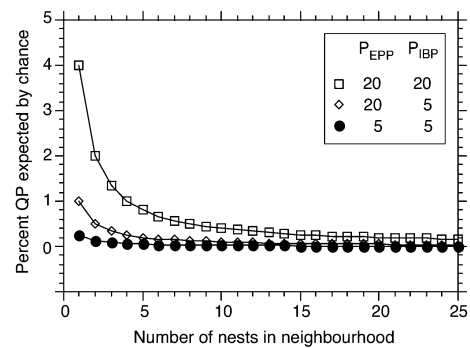

Fig. 1 The expected percentage of eggs in a population that will result from chance QP given the number of nests in the neighbourhood and the percent of eggs in the population that are the result IBP and EPP that are laid in a randomly chosen nest in the neighbourhood. We define a neighbourhood as the nests in close proximity to a focal nest that are likely to be the source of extrapair parentage (EPP or IBP). This model considers, for each egg-laying event, the probability that an egg will be fathered by an extrapair male (P_{EPP}) and laid by an extrapair female (P_{IBP}) in neighbourhoods of different sizes (N). Thus the percent of eggs laid that will attributable to chance QP is (P_{EPP} x P_{IBP})/(N). Model results are shown for a range of values of P_{EPP} and P_{IBP} (as percents of eggs in a population) that might be expected to occur in natural bird populations

parasitic female requires that copulations with the host male enable her to increase her reproductive success by subsequently laying eggs in his nest. A convincing demonstration that QP is adaptive for the host male requires evidence that he benefits from allowing an extrapair female that he has inseminated to lay eggs in his nest.

While all instances of adaptive QP are expected to benefit the parasitic female, they need not always benefit the host male. QP should always be adaptive for females because they control where eggs are laid; a host male cannot force an extrapair female to lay her eggs in his nest. In contrast, we know from many studies that brood parasites often gain access to host nests when it is disadvantageous to the hosts (Gibbons 1986; Møller 1987; Evans 1988; Jackson 1993; Lyon et al. 2002). Thus, a female might use copulations to gain access to a host male's territory, then parasitise his nest without his assistance and at his expense.

How might both parasitic females and host males gain fitness benefits from QP? First, both sexes could gain genetic benefits, manifested by an increase in the quality or viability of offspring (Alves and Bryant 1998). The potential genetic benefits of QP are identical to those proposed for extrapair matings in general (Petrie and Kempenaers 1998; Griffith et al. 2002), and they apply equally to both sexes: (1) enhanced genetic quality of offspring for individuals socially paired with inferior quality individuals, (2) increased genetic variation of offspring, and (3) decreased inbreeding depression for individuals socially paired with close relatives.

Second, both sexes may obtain demographic benefits when QP increases the total number of offspring produced

(Alves and Bryant 1998; Lyon et al. 2002). In contrast to genetic benefits, potential demographic benefits of QP differ for the parasitic female and the host male. For a parasitic female, QP can increase the total number of offspring she produces if it increases her access to host nests when host limitation would otherwise constrain her parasitic fecundity. Such host limitation has been shown to limit the extent of brood parasitism in a few species (Yom-Tov 1980; Lyon 1993b). QP for a female is more likely to be driven by the demographic benefits of increased access to host nests than by the genetic benefits discussed above because a female will gain the genetic benefits from an extrapair mating irrespective of the destination of the extrapair egg (own or host nest), while she gains the demographic benefit only by laying that egg parasitically. From the female's perspective, then, an increased ability to lay parasitic eggs rather than the enhanced genetic quality of her offspring is likely to promote QP.

Adaptive QP for females is thus predicted to be most likely in populations where the fecundity of parasitic females is limited by host availability. Note that, although adaptive brood parasitism (see Yom-Tov 1980; Andersson 1984; Eadie et al. 1988; Lyon 1993a) is a prerequisite for adaptive QP, an explanation for adaptive QP based solely on the benefits of parasitism is inadequate because it fails to explain why a parasitic female mates with the host male that she parasitises. We see this as a critical issue because some previous explanations for QP (Alves and Bryant 1998) confused factors that promote adaptive brood parasitism with those that promote adaptive QP.

The benefits of QP for host males differ from those of parasitic females for both genetic and demographic reasons. Thus, genetic benefits are more likely to favour QP for males than females because males do not lay eggs. QP is the only way that a male can change the maternity of offspring in the brood for which he provides parental care (whereas females can simply use extrapair copulations to gain this increased genetic variation in their brood).

Two factors influence the demographic benefits of QP for host males: (1) the degree to which parasitic chicks affect the survival of host chicks, and (2) the host male's certainty of paternity in his own brood (Lyon et al. 2002). When parasitic chicks have little impact on the number of host chicks produced (e.g., when clutch size is limited by egg production rather than post-hatching parental feeding; Monaghan and Nager 1997), the male gains additional offspring when he sires any of the eggs that a parasitic female lays in his nest. This benefit is diminished when parasitic chicks compete with host chicks and reduce their number. When competition is severe enough, as when a quasi-parasitic chick survives at the expense of a host chick (Lyon et al. 2002), QP may yield no net demographic benefit to a host male. However, the lower a male's certainty of paternity in his own brood, the greater his benefit from QP eggs laid in his nest.

The host male's certainty of paternity in parasitic eggs is also an issue. Even though our focus here is on QP eggs (parasitic eggs that the host male sires), parasitic eggs that a host male fails to sire also affect his net benefits when he trades access to his nest for copulations with the brood parasite. Since it is unlikely that every copulation between a host male and a brood parasite will lead to a QP egg, some fraction of the eggs laid by a brood parasite in the host's nest will not be sired by the host. This is important because all of the eggs the parasite lays in the host nest, both QP and purely parasitic, will compete with host chicks, but the male only benefits from QP eggs. A low probability of siring quasi-parasitic eggs may thus be a major obstacle to the evolution of host-parasite cooperation, particularly when quasi-parasitic chicks have a negative impact on host chicks.

Finally, when brood parasites have nests of their own and begin their own clutches after laying quasi-parasitically, the host male can obtain extrapair offspring via stored sperm that fertilises eggs subsequently laid by the parasitic female in her own nest. This is an important potential benefit that host males could gain from cooperating with brood parasites that has largely been ignored by previous studies (but see McRae and Burke 1996; Lyon et al. 2002). Nonetheless, this benefit is likely to be much more valuable to host males than QP for several reasons (Lyon et al. 2002), and needs to be considered in future studies: (1) unlike QP chicks, extrapair chicks that the host male sires in the parasite's own nest do not compete with the host male's own brood, (2) parasitic chicks often have relatively low survival relative to non-parasitic chicks (Gibbons 1986; Emlen and Wrege 1986; Eadie 1989; Lyon 1993a) so QP eggs would be less likely to yield surviving chicks than eggs that the parasite lays in her own nest, and (3) host males may be more likely to sire eggs that the parasite lays in her own nest (which are typically laid after brood parasitism; Gibbons 1986; Brown and Brown 1989; Lyon 1993b) than the ones she lays in his nest because there is a time lag ($\geq$24 h) between any copulation and the laying of any eggs sired by that copulation.

We have outlined the theoretical fitness benefits of QP for both males and females, but it is currently unknown whether any of these benefits ever actually apply in species with IBP. The paucity of examples of QP in birds could reflect either difficulties in evolving the behaviours required for adaptive QP, or the rarity of ecological conditions that would favour adaptive QP. For example, it might be difficult for extrapair copulations to result in a high enough certainty of paternity by host males in parasitic eggs for QP to ever be advantageous for those males, particularly since eggs are laid well after the copulation that fertilises them. The evolution of adaptive QP may be particularly difficult for males because, as noted above, it will always accompany adaptive QP for females, and it may thus be extraordinarily unlikely that all conditions required for the co-occurrence of adaptive QP in both sexes will be satisfied.

Case histories

In this section we review the published evidence for QP in chronological order, providing details on both the field work (Table 1) and the molecular analyses (Table 2) involved in each study, as well as a critical evaluation of the evidence presented for QP. Because we expect both chance and adaptive QP to be rare in birds, for reasons given above, our approach is decidedly skeptical.

White-fronted bee-eater (*Merops bullockoides*)

In their study of the white-fronted bee-eater, Emlen and Wrege (1986) provide the first published evidence of possible cases QP with two clear examples of extrapair maternity where the pair male could not be excluded as the father. In all, they identified extrapair parentage in 7 of 97 chicks, but were uncertain about its origin in every case (Table 2). Unfortunately, the probability that two randomly chosen individuals from their study population would have the same genotypes was high such that, for each of the seven broods having extrapair parentage, males from two or three other nests in their sample were also identified (in addition to the pair male) as the possible father of the extrapair chicks. Thus, because they could be equally well attributed to simple IBP, not one of the potential cases of QP in this study is unequivocal.

Wrege and Emlen (1987) also attempted to apply the probabilistic modelling approach to identify the source and extent of extrapair parentage in their sample, but found that they had too few mismatches to allow statistical testing. Given their equivocal conclusions about the sources of extrapair parentage in their study colony, it is surprising that they concluded that "All three of these tactics [EPP, IBP, QP] have been observed..." in this species.

Eastern kingbird (*Tyrannus tyrannus*)

McKitrick (1990) also used the probabilistic modelling approach to determine whether EPP, IBP or QP best explained the distribution of extrapair offspring in Eastern kingbirds. She concluded that 'the distribution of exclusions...suggests a model of quasi-parasitism...with secondary females laying one or more eggs in the primary female's nest." In all, McKitrick (1990) identified as many as 20 chicks as potentially resulting from QP (Table 2).

There are, however, several problems with this study. First, the parents were apparently not individually marked. Because kingbirds often intrude on other territories, particularly to harass potential nest predators, including humans (McKitrick 1990), it is difficult to be certain that adults near a nest are actually the parents of that brood. Thus, individual marking is essential to be certain of nest owners in this species. Indeed, the apparently high level of brood parasitism uncovered in this study (Table 2) compared to that in other solitary-nesting passerines (Rowher and Freeman 1989), including other studies of kingbirds (Rowe et al. 2001; see below), suggests that females may have been incorrectly identified as parents here. Second, because the allozymes used for parentage analysis resulted in low probability of exclusion, the father could not be positively identified for any of the 17 extrapair chicks. For example, in the ten families for which McKitrick (1990) presents data, 2–8 males (average 4.2), in addition to the pair male, also matched each of the five chicks that appeared to be the result of either IBP or QP. Thus, on average, there was only a 19% (1/5.2) chance that the genetic father of these chicks was correctly identified. For the remaining 12 extrapair chicks where parental mismatch was completely ambiguous (IBP, QP or EPP), 1–10 other males (average 7.8) in the sample, in addition to the putative father,

Table 2 Molecular evidence for quasi–parasitism in birds. See Table 1 for background details and references. Where possible, statistics are presented as mean±SD bands/individual

Species	Analysis	Markers	Nestlings: n (% of total)			
			Total	EPP	IBP	QP
White-fronted bee-eater	Allozyme	3 loci (2–5 alleles each)	97	2–4 (2–4)[a]	0–5 (0–5)[a]	0–4 (0–4)[a]
Eastern kingbird	Allozyme	3 loci (2–5 alleles each)	60	1–13 (2–22)[a]	0–17 (0–28)[a]	0–17 (0–28)[a]
Zebra finch	Multilocus	2 probes; 20–50 bands/individual	92	2 (2.2)	7 (7.6)	1 (1.1)
Bearded tit[b]	Multilocus	1 probe; 26±4.8 bands	141[c]	27 (19.1)	5 (3.5)	7 (5.0)
Penduline tit	Multilocus	1 probe; 11.5±7.7 bands	187	14 (7.5)	1 (0.5)	2 (1.1)
Water pipit	Multilocus	1 probe; 27.2±6.2 bands[e]	1052	55 (5.2)	5 (0.5)	5 (0.5)
Sand martin	Multilocus	1 probe; 20.0±3.5 bands	167	23 (13.8)	3 (1.8)	4 (2.4)
Black-capped chickadee	Multilocus[d]	2 probes; no. of bands not reported	359	32 (8.9)	7 (1.9)	2 (0.6)
Kentish plover	Multilocus	1 probe; no. of bands not reported	170	1 (0.6)	0	2 (1.2)
Common sandpiper	Multilocus	1 probe; no. of bands not reported	53	1 (1.9)	0	3 (5.7)
House sparrow	Multilocus	1 probe; 29.7±1.2 bands in adults	123	25 (18)	2 (1.4)	1 (0.8)
Barn swallow	Microsatellite	3 loci; 78, 125 and 66 alleles	674	120 (17.8)	0	17–18 (2.6)[f]

[a] Parentage uncertainties due to methods of molecular analysis
[b] Data from nests in colonies only; in 12 solitary nests with a total of 46 chicks there were no instances of extrapair parentage
[c] Estimated from their Fig. 1
[d] Plus 2 microsatellites and a single locus minisatellite probe to confirm extrapair parentage in the final year of study (see text)
[e] Plus an additional probe (21.6±3.1 bands) for one ambiguous case
[f] Number not stated in paper

matched each chick. Thus all 17 of these possible examples of QP are just as likely to have been examples of IBP, where the real father and mother were the attendants at another nest. Third, the sample size of families in this study is too low to provide enough statistical power to reasonably evaluate the results of the probabilistic model. Though EPP was rejected statistically as a general explanation for the distribution of possible exclusion types (i.e. both male and female, male only, female only, and ambiguous), both an IBP model and a QP model failed to be rejected. Thus, McKitrick (1990) remarked that "More accurate methods of determining the source of stray genes, such as DNA fingerprinting...will be necessary to confirm the occurrence and extent of both forms of parasitism in eastern kingbirds".

Such a study has recently been published (Rowe et al. 2001), using multilocus DNA fingerprinting to analyse parentage of 64 nestlings from 20 broods. In that study, all adults were colour-banded and their mating status unequivocally determined by careful observations, but no evidence of QP or IBP was found. In all, 27 nestlings (42%) were identified as extrapair but none of these (nor any other nestlings) were mismatched with the attending female.

Zebra finch (*Taeniopygia guttata*)

Birkhead et al. (1990) provided the first report of QP based on multilocus DNA fingerprinting. Their genetic evidence for extrapair maternity is supported by parasitised clutches having, on average, one more egg (6.0±0.82 SD, $n=7$) than unparasitised clutches (5.0± 0.95 SD, $n=12$). Remarkably, "One of the parasitic nestlings (I268) was significantly more closely related to the nest-holding male than expected for a non-relative. There is therefore the possibility that I268 is the result of quasi-parasitism...In the absence of data other than from fingerprinting, we cannot in this instance prove paternity. The father must, however, have been at least related to the nest holding male. We cannot disprove the possibility that the true father was a first order relative of the putative father" (Birkhead et al. 1990, p 320). While this is a reasonable and cautious conclusion, it was based on the analysis from a single multilocus probe and, as we noted above, such results are easy to misinterpret. Thus, we agree with Birkhead et al. (1990) that there is not enough evidence to be certain that this is a case of QP.

Bearded tit (*Panurus biarmicus*)

Hoi and Hoi-Leitner (1997) reported that 3.7% of 187 bearded tit offspring could be attributed to QP. Given the relatively large number of bands scored and the methods of paternity analysis used in this study (Table 2), we have some confidence in these results.

Interestingly, all instances of EPP, IBP and QP uncovered in this study occurred in small colonies ($n=32$ nests) but not in solitary nesters ($n=12$ nests). Rates of QP in just the colonial nests are almost the highest so far reported in the literature (Table 2), and the fact that QP is more common than IBP suggests that QP was not accidental.

Penduline tit (*Remiz pendulinus*)

Schleicher et al. (1997) report that one nestling in each of two penduline tit broods were most likely the result of QP. However, this species has a sequentially polygynandrous social mating system with "one male paired with up to four females and one female can have up to three males during one breeding season." (Schleicher et al. 1997). Thus, males build and defend nests rather than territories, and females seem to choose males, at least in part, on the quality of their nest. During or immediately after egg-laying, the male deserts and immediately builds and defends another nest to attract another female, and so on. Thus, it is quite possible that the two apparent examples of QP were simply the result of this type of rapid mate switching.

Water pipit (*Anthus spinoletta*)

In a large-scale study of the socially monogamous water pipit, Reyer et al. (1997) found that one nestling in each of five broods could be attributed to QP. This level of QP was matched by the incidence of IBP, but dwarfed by the frequency of EPP (Table 2). In all, 54 adults were parents in more than one of the broods studied, but it is not reported whether the five supposed cases of QP were independent events or were fathered by the same male(s) in subsequent broods (Reyer et al. 1997).

Thus, QP was a very rare event in this population, most likely resulting from females not restricting their activities to their own territories (Reyer et al. 1997). Instead, females regularly foraged on adjacent territories, and at communal feeding places that they had to cross several territories to access, thereby increasing the likelihood that they would encounter a nest to dump an egg in. These forays would also have increased the likelihood that an extrapair copulation with another male may occasionally have resulted in an egg laid in his nest. The impressive sample size of offspring surveyed by Reyer et al. (1997) certainly provided plenty of opportunity for the detection of rare phenomena.

Sand martin (*Riparia riparia*)

In the colonial breeding sand martin, Alves and Bryant (1998) identified QP as the source of one chick in each of four nests (Table 2), three of which also had an EPP chick. None of the nests with either QP or IBP chicks had an anomalous laying sequence that would suggest the presence of a supernumerary egg. Thus, Alves and Bryant

(1998) suggested that the parasitic female must have removed a host egg each time a parasitic egg was laid and, indeed, some recently laid eggs were found discarded beneath some burrows.

Because QP occurred in twice as many broods as IBP and never in nests that also had IBP eggs, Alves and Bryant (1998) argued that adaptive QP was more likely than chance QP in this population. However, in this species, the opportunity for chance QP is particularly high as nest burrows are often located within a metre of each other and the level of EPP is relatively high (Table 2). Thus, if females that engage in EPCs with neighbouring males are most likely to dump eggs in a nearby burrow, chance QP would result. It is also possible that the level of QP reported for this population is in error, as Alves and Bryant (1998) note "that further behavioural evidence is needed to warrant detailed consideration of serial QP." and that "Attribution errors cannot...be excluded altogether, mainly because behavioural observations were incomplete at nests that later showed QP. These allow for undetected mate changes prior to rearing."

A recent study of this species in Hungary found no evidence for QP, with 80 offspring from 22 families and their putative parents genotyped at three microsatellite loci (H. Nicholls personal communication). IBP occurred in 8 of 22 broods, accounting for 9 (11%) of 80 offspring and EPP occurred in 8 of 20 broods, accounting for 15 (24%) of 63 offspring. Thus, even with relatively high levels of EPP and IBP, and nests in close proximity, no QP was detected (H. Nicholls personal communication).

Black-capped chickadee (*Poecile atricapillus*)

Otter et al. (1998) detected a possible case of QP in the final year of their study of black-capped chickadees. In that year, 9 (10%) nestlings were attributed to EPP, 7 (8%) to IBP and 2 (2%) to QP (*n*=87 nestlings in 16 nests; Ken Otter, personal communication). Both instances of QP occurred in the same brood of eight nestlings and the parentage of all nestlings in this brood was confirmed with both single locus and microsatellite markers. In this brood, only two nestlings were the offspring of the social mother and father, whereas six were the offspring of the neighbouring female (with two sired by the social father, three by the parasitic female's social mate and the remaining nestling sired by the female's original mate at the start of that breeding season). Because the IBP and QP nestlings "hatched several days before the within-pair young", Otter et al. (1998) conclude that these six nestlings were most likely the result of "a takeover of a partially laid clutch" and thus not QP as we have defined it here.

Kentish plover (*Charadrius alexandrinus)* and common sandpiper (*Actitis hypoleuca*)

A recent study of extrapair paternity in three shorebird species (Blomqvist et al. 2002) reports QP in both the Kentish plover and the common sandpiper (Table 2), though not in the western sandpiper (*Calidris mauri; n*=61 chicks). These five apparent instances of QP were not accompanied by any examples of IBP, and EPP was rare (Table 2). Thus, every extrapair female laid each of their eggs in the nest of the male who fertilised the egg.

Since no supernumerary eggs were found in any of the parasitised nests (Blomqvist et al. 2002), either (1) males removed an egg laid by their social partner to compensate for the QP egg, (2) the parasitic female removed an egg when laying one of her own, or (3) the pair female adjusted her clutch size as a result of the extra egg appearing in her nest. However, none of these scenarios seems likely as neither intact egg removal or clutch size adjustment has been reported in these or any other waders. Moreover, when an egg is removed, the male suffers the loss of an egg laid by his pair female, with whom he copulates regularly and with whose offspring his likelihood of paternity is 93–98% (based on EPP levels reported in Blomqvist et al. 2002), in favour of an egg laid by a female with whose offspring his certainty of paternity must be appreciably lower. Thus, the indirect benefits to be gained from males indulging in QP must be extremely high such that males are prepared to accept a potential reduction in paternity, as well the potential costs (reduced maternal care or divorce) if the pair female were to discover her partner consorting with other females. Such high indirect genetic benefits have not been documented in many other species and where they do occur are generally found in tandem with relatively high rates of EPP (Griffith et al. 2002).

A more recent study of the common sandpiper in Scotland (Mee et al. 2004), which surveyed twice as many families, scoring more than twice as many multilocus DNA fingerprint bands in each individual, found no evidence of either QP or IBP.

House sparrow (*Passer domesticus*)

Vaclav et al. (2003) report one case of QP in house sparrows (Table 2). However, they provide no information about how putative parents were assigned so it is impossible to assess the reliability of the apparent maternal mismatches. More recent analyses using three microsatellite loci to assess parentage in families collected the following year by the same study suggests that this apparent case of QP is more likely to be a case of sampling error (S. Griffith, unpublished data).

Parentage in the house sparrow has also been studied by six other research groups using multilocus, single-locus, and microsatellite DNA profiling (Wetton and Parkin 1991; Cordero et al. 1998; Griffith et al. 1999; Whitekiller et al. 2000; Veiga and Boto 2000). In these

studies, a total of 1,442 offspring have been scored from 471 families revealing that 10.1% of offspring had extrapair parentage, but not a single case of either IBP or QP.

Barn swallow (*Hirundo rustica*)

Møller et al. (2003) report as many as 17 cases of QP distributed among five broods of the barn swallow (Table 1). While the molecular analysis in this study is exemplary (Table 2), the level of QP (c. 3.4 nestlings per brood in nests with QP offspring), and the absence of IBP in this population, suggests to us that these may represent examples of rapid mate switching or misidentification rather than QP. Moreover, the authors present no further data on these examples so it is unknown whether the mothers were nearby neighbours in this "extremely high density" population (Møller et al. 2003) or indeed whether they might have been previous mates of the sires for earlier broods in the same season. Further work is needed to reveal whether this is a case of adaptive QP as it appears to be or might be explained by a simpler mechanism.

Conclusions

Studies of 12 bird species report molecular evidence that could be considered as evidence for the occurrence of QP. Our close examination of these studies reveals that unequivocal evidence for QP is slim indeed. For example, there is not sufficiently reliable molecular evidence to be certain of the existence of QP in the white-fronted bee-eater, eastern kingbird, or zebra finch. In the black-capped chickadee molecular evidence for QP was shown to be more likely explained by mate switching when the behavioural data were examined in light of reliable parentage analysis (Otter et al. 1998). Likewise, it is difficult to conclude that there is evidence for QP in the penduline tit (Schleicher et al. 1997) due to its complex social breeding system with rapid mate switching of both male and female partners.

QP also seems highly unlikely in the house sparrow, Kentish plover and common sandpiper, for both theoretical and methodological reasons (see also Griffith and Montgomerie 2003). Thus, we suggest that these examples need to be substantiated with better molecular analyses coupled with solid behavioural evidence. In the bearded tit, sand martin, barn swallow and alpine water pipit, the molecular data is consistent with QP, although in no case was this accompanied by clear behavioural evidence in support of QP (Hoi and Hoi-Leitner 1997; Alves and Bryant 1998; Reyer et al. 1997). Therefore, it is possible that, at least in some cases, apparent QP may actually be the result of mate switching or misidentification. Overall, however, the studies by Alves and Bryant (1998) and Hoi and Hoi-Leitner (1997) provide the best evidence for QP, and in both species it is possible to imagine a theoretical framework for an adaptive explanation (e.g. both EPP and IBP are regular alternative breeding strategies in these species). The barn swallow data (Møller et al. 2003) are simply too sketchy to be critically evaluated but, taken at face value, they provide the clearest molecular evidence for QP.

It is worth noting that more recent studies of eastern kingbird, sand martin, common sandpiper and house sparrow, in each case based on larger samples of families and superior molecular analyses (better markers or more minisatellite probes), have failed to find any examples of QP or even IBP. This raises the possibility that these examples of QP are artefacts of the techniques used and highlights the general inadequacy of allozyme and multilocus markers to reveal complex parentage patterns, like QP. Parentage analyses with low statistical power leave open the possibility that a real parent will be incorrectly excluded or a false parent matched to an offspring.

Our review of the case studies reveals that QP is, at best, a very rare phenomenon in birds, far less frequent than the recent literature would suggest. The rarity of QP in birds seems less surprising when we consider the relatively complex and unusual set of conditions required for the evolution of adaptive QP. Even in those species where these requirements seem to be adequately met, no evidence of QP has been found (McRae and Burke 1996; Lyon et al. 2002). For this reason we propose a strict set of criteria for demonstrating the existence of QP in future studies of birds: good evidence of parasitic egg laying, molecular analysis of parentage using either unequivocal microsatellites or two or more different multilocus probes with >40 bands scored in focal individuals, careful protocols to ensure that samples have been correctly identified, molecular sexing of adults to establish that parental samples are correctly assigned, and clear behavioural evidence that the putative parents are socially associated with the focal offspring, and that social parents have not changed, (i.e. no mate switching has occurred during the breeding attempt).

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