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Cross-fostering as a means to study kin recognition

JILL M. MATEO* & WARREN G. HOLMES†

*Department of Psychology, Cornell University
†Department of Psychology, University of Michigan

Cross-fostering techniques in which infants are taken from their genetic parents and reared by unrelated foster parents have been widely used in behavioural research to investigate several developmental questions. Here we address some of the central issues involved in using cross-fostering to study kin recognition where it has been applied frequently. We do so by first outlining general principles of recognition mechanisms, and then presenting three different cross-fostering designs that allow researchers to examine specific details of the development of recognition abilities. Our discussion focuses on kin recognition, although the transfer of young between litters, broods or clutches is a commonly used tool for other behavioural questions, including vocal development in primates (Owren et al. 1993) and birds (Medvin et al. 1992; Payne et al. 1998), the effects of experience and genes on parenting or aggression (Breed & Rogers 1991; Maestripieri et al. 2000; Kölliker & Richner 2001), the influence of parents and offspring on pro-visioning rates (Gray & Hamer 2001), sexual imprinting (ten Cate & Vos 1999) and recognition of parasitic eggs by avian hosts (Bischoff & Murphy 1993).

In kin recognition research, cross-fostering has been used to disentangle the effects of relatedness and prior association on kin recognition, in which 'relatedness' refers to factors that correlate with genes identical by descent (e.g. phenotypic resemblance) and 'prior association' refers to direct interactions between individuals that result in familiarity with each other's traits. Kin recognition is mediated by two learning-based mechanisms in many species (see Holmes & Sherman 1982). First, in the prior-association mechanism (PA), an animal learns the cues of individuals that it encounters during early development such as parents or siblings and later distinguishes between these 'familiar' and 'unfamiliar' conspecifics. Second, in the

phenotype-matching mechanism (PM), an individual learns the phenotypic cues of its rearing associates and/or its own cues ('kin referents') and stores a representation of these traits in memory as a 'kin template'. Later, individuals compare the phenotypes of unidentified conspecifics to this template ('phenotype matching'), with the degree of match indicating their degree of relatedness (Getz 1981; Lacy & Sherman 1983). For those species in which there has been over evolutionary time a reliable correlation between shared rearing environments and kinship (the PA mechanism) or in which phenotypic and genotypic resemblance covary (the PM mechanism), individuals may gain inclusive fitness benefits that are mediated by these recognition mechanisms (Sherman & Holmes 1985), although they may not have evolved to mediate nepotism specifically (Barnard 1990).

To simplify our discussion, we will not address 'spatially based' or 'recognition allele' mechanisms of recognition; interested readers should see Hamilton (1964), Alexander (1979), Beecher (1982) and Holmes & Sherman (1982) for discussions of these mechanisms. Some kin recognition researchers refer to 'direct' recognition, when discrimination among kin is based on traits they express, and 'indirect' recognition, when discrimination is based on contexts, such as spatial location, rather than on individuals themselves (see Waldman et al. 1988; see also a discussion of 'direct and indirect co-bearer discrimination' by Barnard 1990). We focus on PA and PM as mechanisms mediating recognition, both of which would be considered forms of direct recognition. We do so because the aim of our paper is to address the fostering methods used to study behavioural development in general, rather than to present conceptual advances in kin recognition theory.

Both PA and PM assume that learning occurs when only kin are present, usually during early development, as would often be the case in burrows or nests in which single litters, broods or clutches are reared. In addition, PM requires a correlation between genotypic and phenotypic similarity (e.g. Greenberg 1979) such that individuals are recognized as kin if their phenotype matches favourably with an acquired kin template (Getz 1981; Reeve 1989; Sherman et al. 1997). Both mechanisms involve a comparison between encountered phenotypes

Correspondence and present address: J. M. Mateo, the Committee on Human Development, 5730 South Woodlawn Avenue, University of Chicago, Chicago, IL 60637, U.S.A. (email: jmateo@uchicago.edu). W. G. Holmes is now at the Department of Psychology and The Center for Ecology and Evolutionary Biology, 308B Pacific Hall, 5289 University of Oregon, Eugene, OR 97403-5289, U.S.A.

and stored representations, yet their outcomes differ. The PA mechanism permits recognition of only previously encountered (familiar) individuals, whereas the PM mechanism, probably through a process related to stimulus generalization, also permits recognition of never-before-encountered kin (e.g. paternal half-siblings reared apart) or discrimination among kin originally encountered in a 'mixed' environment comprising unequally related individuals (e.g. full and maternal half-siblings reared together). As Waldman (1987) was the first to note, PA and PM may be differentiated more by ecological and contextual considerations than by neural processes. None the less, whether PA and PM are discrete mechanisms at the physiological level is not critical to our focus on cross-fostering because our interest is in how effects of early experience are manifested at the behavioural level. We do note, however, that the perceptual processes by which animals are matched to recognition templates differs between PA and PM (exact match to the template versus generalization from the template as a gestalt representation of 'kin', respectively), as might the ways in which referents are encoded in templates (e.g. as individuals or as exemplars or prototypes). We view PA and PM as recognition mechanisms not only with distinct functional outcomes, but also with distinct physiological and perceptual processes mediating how referents in the templates are used to identify conspecifics (see also Hamilton 1964; Holmes & Sherman 1982; Waldman 1987; and see Barnard 1990 for a variant on this distinction). Therefore, in our discussion we treat PA and PM as proximately and functionally different mechanisms, although our analysis of cross-fostering as a means to study kin recognition would not be altered if one considers PA and PM to be more physiologically similar than different.

Depending on the research question, several decisions are required to use cross-fostering properly, including, for example, how many young to transfer and whether to transfer young reciprocally between two (or more) sets of parents. Below we discuss three designs that can be used to evaluate the contribution of PA and PM to the development of recognition abilities and to identify the individuals whose cues are assimilated into recognition templates. These cross-fostering designs can also be applied to other areas of behavioural development, such as sexual imprinting, vocal development or the effects of genes and experience. Our goal in detailing the methods and interpretation of cross-fostering designs is to clarify several issues in the cross-fostering literature, especially when our views differ from those of others (cf. Todrank & Heth 2001). We make four assumptions that collectively restrict the situations to which our discussion applies. First, we assume that the recognition abilities of fostered animals are tested in simultaneous or sequential discrimination tasks with either conspecifics (e.g. paired-encounter tests) or just their cues (e.g. odours, vocalizations). Second, we assume for heuristic purposes that in paired-encounter tests one individual's recognition abilities can be assessed independently of the other individual's, which may not always be true (see Holmes 1986a). Third, we assume that kin labels are not transferred among rearingmates (e.g. acquired from the mother, nestmates or the nest itself) and that individuals bear only their own labels at the time of testing, although this assumption is sometimes incorrect (e.g. Aldhous 1989), especially among eusocial insects (Breed & Bennett 1987). When this assumption is violated, interpreting cross-fostering studies may be difficult (e.g. Smith et al. 1994). Finally, we make the simplifying assumption that the experience young have before they are cross-fostered, including prenatal experience, does not influence recognition mechanisms, although this is not always true (e.g. Hepper 1991), and may render postnatal cross-fostering ineffective as a means to disentangle the effects of relatedness and prior association on kin recognition. In addition, whether one uses preference tasks, rates of social interactions or mate choice behaviours as an index of recognition, it is important to note that a lack of differential treatment cannot be interpreted as a lack of recognition ability (Gamboa et al. 1991) and that differential treatment need not imply that kin will inevitably be treated preferentially (Holmes 2001). We will use 'litter' in our discussion, although 'brood', 'clutch' and other similar terms could be substituted.

Multiple-transfer Design: Distinguishing between Prior Association and Phenotype Matching

Recognition of unfamiliar kin (i.e. kin reared apart from each other) by PM can be studied empirically by determining whether normally reared individuals can recognize their unfamiliar kin (e.g. paternal half-siblings), or by cross-fostering littermates at birth and later testing their ability to recognize their unfamiliar full siblings. If normally reared animals (i.e. non-cross-fostered young) recognize their unfamiliar kin in the absence of prior association, phenotype matching is suggested, although additional work would be needed to verify this inference. For example, one would need to manipulate recognition templates and find predictable changes in discrimination patterns based on how templates were manipulated. Cross-fostering is the preferred method for revealing discrimination among equally familiar individuals (e.g. genetic and foster siblings reared together) or unfamiliar individuals (e.g. older full siblings and nonkin) and for determining the kin referents used for recognition (see below).

In the widely used reciprocal, multiple-transfer design, two or more individuals from one litter and an equal number from another litter are exchanged reciprocally shortly after birth (Fig. 1). PM would be suggested if fostered animals, after some period of development, responded differentially to their unfamiliar kin and unfamiliar nonkin or their cues (in Fig. 1, test B1 with B4 and C1). By presenting subjects with unfamiliar stimuli only, one can avoid confounding discrimination based on relatedness with discrimination based on differential familiarity. One could also test B1 with equally familiar individuals that vary in relatedness (e.g. genetic and foster siblings; in Fig. 1, test B1 with B2 and A2), and discrimination would suggest PM. However, the lack of discrimination could be hard to interpret vis-à-vis PM because the potency of familiarity may mask PM. Recognition based on PA would

be demonstrated if fostered animals discriminated between their familiar and unfamiliar kin (e.g. in Fig. 1, test B1 with B2 and B3) or between their familiar and unfamiliar nonkin (e.g. A1 and A3). For these PA tests, both stimulus animals should be either related or unrelated to the fostered animal to control for the possibility of discriminating on the basis of genetic relatedness rather than familiarity.

The reciprocal, multiple-transfer design (Fig. 1) is sufficient for evaluating the effects of PA and PM on kin recognition, because young are exposed to kin and nonkin cues that may later be used as referents for recognition. In fact, by transferring multiple individuals from a litter, each of them can later serve as subjects in recognition tests, thus reducing the total number of litters required for a study. In this case, however, one should test for litter effects (greater between-litter variation than within-litter variation) to ensure that subjects from the same litter represent independent data points (Gamboa et al. 1991).

Multiple-transfer designs have a long history in kin recognition studies, particularly in the early 1980s when the mechanisms of recognition were first explored (e.g. Beecher et al. 1981; Waldman 1981; Davis 1982; Holmes & Sherman 1982; Porter et al. 1983; Gavish et al. 1984). PM has been demonstrated in many taxa, including fish (e.g. Brown et al. 1993; but see Griffiths & Magurran 1999), anurans (e.g. Waldman 1981), invertebrates (e.g. Buckle & Greenberg 1981; Getz & Smith 1986), birds (e.g. Bateson 1980) and mammals (e.g. Porter et al. 1983; Mateo & Johnston 2000; Mateo 2002). Many of these studies also demonstrate effects of PA on discrimination, showing that cues of foster siblings were learned. Historically, many researchers have proposed that animals use different recognition mechanisms in different functional contexts, such as mate choice and nepotism (including parental care), and that the mechanisms used can change across time (Sherman & Holmes 1985; Waldman 1986; but see Alexander 1990). So with careful empirical design, the relative importance of PA and PM to discrimination can be evaluated.

Single-transfer Design: Demonstrating Self-referent Phenotype Matching

A central challenge in PM research is to identify the kin referents that contribute to kin templates (Hauber & Sherman 2001). When more than one individual from a litter is transferred to another nest, as occurs in a multiple-transfer design (Fig. 1), the recognition templates of those transferred individuals may include cues from several potential referents, including their (1) foster siblings, (2) foster parent, (3) genetic siblings that were transferred with them, and/or (4) themselves. We believe that different cross-fostering designs must be used to determine whether particular referents are assimilated into kin templates. In particular, this is true for studies of self-referent PM.

Self-referent PM occurs when an animal learns some aspect of its own phenotype, incorporates this into its kin template and uses its own phenotype as a referent to identify its relatives (Holmes & Sherman 1982). The existence of this mechanism has been debated on theoretical and empirical grounds, particularly the likelihood of it being used in nepotistic contexts (Alexander 1991; Sherman 1991). Self-matching could mediate recognition when multiple paternity occurs within litters, for example, to discriminate between equally familiar full and maternal half-siblings. It could also operate when individuals encounter unfamiliar kin for the first time, such as when paternal half-siblings initially meet (Holmes 1986a) or if siblings are not encountered until after dispersal (e.g. cell-mediated settlement patterns in larval marine tunicates; Grosberg & Quinn 1986). Self-matching may also operate in other functional contexts such as species recognition in brood parasites (e.g. Hauber et al. 2000).

Because of recent interest in possible examples of self-referent PM (fish: Olsén et al. 1998; birds: Petrie et al. 1999; Hauber et al. 2000; Shorey et al. 2000; humans: Jacob et al. 2002), we will detail the methods necessary to investigate it (see Fig. 2). To study self-matching, animals should be cross-fostered so that they are reared without exposure to kin cues (other than their own) to prevent 'social learning' from their relatives (Alexander 1991); that is, only one animal per litter should be transferred to a foster litter (e.g. B1 in Fig. 2). After some period of development, if the
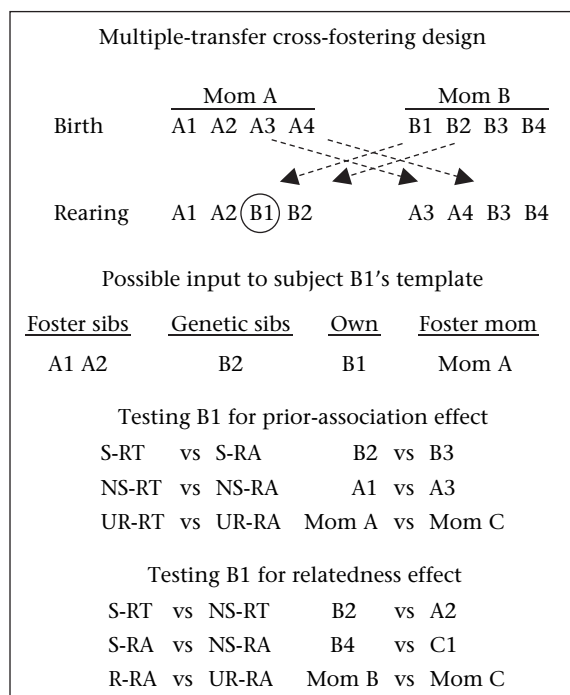

Figure 1. A schematic drawing of the multiple-transfer cross-fostering design in which two (or more) individuals from one litter and an equal number from another litter are exchanged (see dashed lines) reciprocally between litters. By way of example, the discrimination abilities of B1 can be tested to determine the developmental basis of kin recognition. For example, B4 and C1 could be presented to B1 to test the effect of relatedness on B1's discrimination abilities. The litters from which some individuals (e.g. Mom C, C1) derive are not shown. S = sibling; NS = nonsibling; R = related; UR = unrelated; RT = reared together; RA = reared apart.
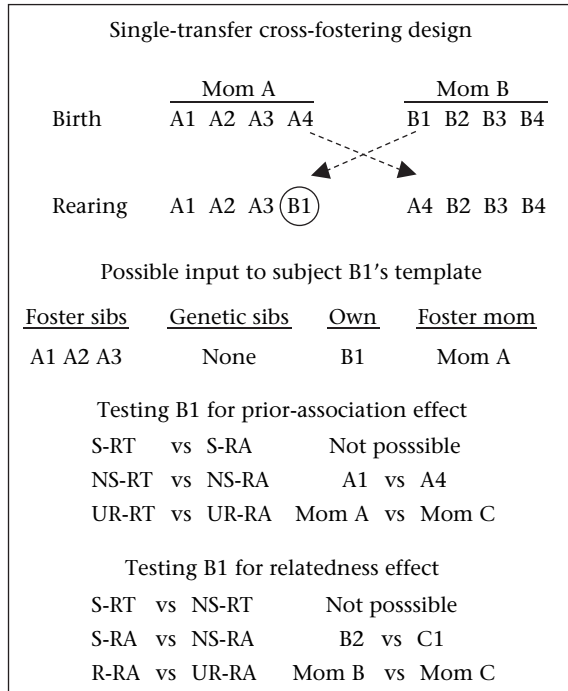
Figure 2. A schematic drawing of the single-transfer cross-fostering design in which one individual from one litter and one from another litter are exchanged reciprocally between litters. As explained in the text, the discrimination abilities of B1 can be tested to determine whether, among other possibilities, self-referent phenotype matching occurs. The litters from which some individuals (e.g. Mom C, C1) derive are not shown. Abbreviations as in Fig. 1.

fostered animal distinguishes between its unfamiliar kin and unfamiliar nonkin (that are also unrelated to the fostermates; e.g. in Fig. 2, test B1 with B2 and C1), this would demonstrate self-matching, because the fostered individual would have to use its own cues as a basis for discrimination (again assuming no prenatal learning of kin cues). Testing the fostered individual with cues from the unfamiliar kin of its foster siblings and its unfamiliar genetic kin (e.g. in Fig. 2, test B1 with A4 and B4) cannot reveal self-matching. This is because discrimination, if it occurred, could be based on either a self template or one that included only unrelated nestmates' phenotypes. If discrimination did not occur (i.e. B1 did not differentiate between B2 and C1), it could mean that (1) the fostered individual cannot make the discrimination under any test conditions or (2) the fostered individual incorporated into its kin template the phenotypes of both its foster siblings and itself (e.g. Aldhous 1989) and the two sources were combined in a way that precluded discrimination based on self-matching. We note here that like the multiple-transfer design (Fig. 1), the single-transfer design (Fig. 2) can also be used to study recognition based on the PA mechanism by determining whether the fostered animal can discriminate between its familiar and unfamiliar nonkin (in Fig. 2, test B1 with A1 and A4).

One reason that the single-transfer design has not been commonly used in studies of behavioural development is because of sample size constraints, that is, one subject/litter. However, some studies have used this design to examine self-matching, and we describe a few of them. First, Buckle & Greenberg (1981) studied nestmate recognition in captive sweat bees, *Lasioglossum zephyrum*, and reared a lone female among a group of sisters that were unrelated to her. As nest guards, these lone females allowed entry by the unfamiliar sisters of their unrelated nestmates and rejected their own unfamiliar sisters (see their Figure 2). The authors concluded that bees learned only the odours of their unrelated nestmates and that there was no evidence for self-matching (but see an alternative interpretation, based on the number of conspecifics encountered during development, in Getz 1982). Nestmates' cues may serve as adequate referents for *L. zephyrum* to recognize unfamiliar intruders because in nature females establish colonies near their natal nest, which generates neighbourhoods of related colonies (Kukuk & Decelles 1986) in which phenotypic similarity would be high. Second, in research on female golden hamsters, *Mesocricetus auratus*, Mateo & Johnston (2000) cross-fostered females singly on the day of birth, and, as adults, these fostered females discriminated between the odours of their unfamiliar kin and unfamiliar nonkin. Because fostered hamsters were reared apart from all of their kin since birth, the only way they could recognize the odour of their unfamiliar relatives was if they compared the test odours to their own, thus demonstrating self-referent PM. The function of self-matching in hamsters remains unknown (Mateo & Johnston 2003). The single-transfer design has also been used to investigate kin recognition in house mice, *Mus domesticus*. Using a laboratory strain, Aldhous (1989) reported that transferred individuals learned self cues that, in paired-encounter tests, may have played a modest role in mediating behaviour towards unfamiliar kin. Smith et al. (1994), working with wild house mice, also found suggestive evidence that self cues were learned but argued that this learning was geared towards group-member discrimination rather than kin discrimination.

A variation of the single-transfer design involves removing an individual from its kin and rearing it isolated from all conspecifics. For example, Blaustein & O'Hara (1981, 1983) found that Cascade frog, *Rana cascadae*, tadpoles reared alone since the egg stage later preferred unfamiliar kin over unfamiliar nonkin in preference tests. The authors hypothesized that PM would be more important for kin recognition in this species than PA, as clutches from multiple females commingle, thus creating groups of kin and nonkin eggs. Removal of the maternal egg jelly does not impair kin recognition in *R. cascadae* (Blaustein & O'Hara 1982), suggesting to us that tadpoles may use their own cues, rather than those derived from their mother, to recognize their siblings (perhaps unlike *Bufo americanus* tadpoles; Waldman 1981). Yet an experiment combining single transfers and egg-jelly removal would be necessary to conclude a self-matching mechanism. Getz & Smith (1986) also used isolation rearing to study self-matching and found that worker honeybees, *Apis mellifera*, could use their own phenotype to discriminate between unfamiliar full (super) and half-sisters.

Todrank & Heth (2001) claim that their work (Heth et al. 1998) with cross-fostered male golden hamsters

demonstrates self-referent PM. We question this claim because the investigators fostered halves of litters 7 days after birth, such that fostered animals were reared in litters with at least one genetic sibling, which means that subjects encountered kin cues other than their own both before and after cross-fostering. Transferring two or more individuals between litters shortly after birth is suitable to determine whether animals can discriminate conspecifics based on PA, PM or both, as discussed above for Fig. 1. However, a multiple-transfer design is not appropriate to test for self-matching because fostered animals may assimilate into their recognition templates the cues of their kin that were fostered with them, as well as their own cues, either of which could later serve as a basis for comparison (Alexander 1991; Mateo & Johnston 2000). We maintain that the results of Heth et al. (1998) indicate the effects of both PA and PM on the discrimination abilities of male *M. auratus*. We do not believe, however, that their results can address the self-matching issue because multiple siblings were transferred together between litters.

Whether a multiple- or single-transfer design is used to study PM, it may be important to consider the kin:nonkin number, that is, the number of related and unrelated individuals in a foster litter that could contribute to a recognition template (Buckle & Greenberg 1981). If each individual's phenotype more or less equally contributes to a focal individual's template, then the focal's template might be weighted in proportion to the kin:nonkin number that it experienced during development. Similarly, if each phenotype is weighted equally, then the focal's ability to use self-referent PM may be masked if it were reared among many nonkin. For example, Getz (1982) used a genetic model to show that whether a female hymenopteran could recognize her unfamiliar sisters would depend on the size of the group in which she was reared and thus the number of related and unrelated genotypes she experienced during development.

Nonreciprocal Transfer Design: Determining Whether Parents' and Siblings' Cues are Both Incorporated into Recognition Templates

In the designs that we have discussed (Figs 1, 2), fostered individuals that receive parental care are exposed to the cues of their agemates and to those of their foster parent(s). Several studies have demonstrated that both related and unrelated nestmates' cues are assimilated into recognition templates (e.g. Buckle & Greenberg 1981; Holmes 1986b; Hepper 1987; Mateo & Johnston 2000), but little is known about whether a fostered individual incorporates into its recognition template the cues of its agemates, of its rearing parent(s), or both (but see Hepper 1987). For instance, Holmes (1986b) used a single-transfer design in a study of female Belding's ground squirrels, *Spermophilus beldingi*, to determine whether a female reared only with agemates unrelated to her would later recognize her unfamiliar sisters. After rearing, single-transferred females were less agonistic with their unfamiliar sisters than with unfamiliar, unrelated females in paired-encounter tests, which suggests discrimination based on self-matching, an ability that might be important given the frequency of multiple

paternity in *S. beldingi* litters (Hanken & Sherman 1981). However, Holmes (1986b) noted that because some fostered females were tested with a sister that had been reared by their genetic mother, it was possible that one female in each test pair learned kin cues from her mother rather than herself (see also Alexander 1990).

Despite the potential importance of foster parents' cues in template formation (see below), few studies have considered the role of these cues, which can be pursued empirically with a nonreciprocal cross-fostering design (Fig. 3). In this design, a fostered individual (e.g. B1 in Fig. 3) is exposed to several distinct sets of phenotypes, including cues from two families that are unrelated to it: foster siblings (A1, A2) and the foster mother (Mom C). If B1 had incorporated its foster mother's cues into its template, B1 should be able to discriminate between Mom C's genetic offspring and offspring unrelated to her, both of which are unfamiliar to B1 (e.g. in Fig. 3, test B1 with C1 and D1). If B1 had incorporated the cues of its foster siblings into its template, B1 should be able to discriminate between unfamiliar kin of its foster siblings
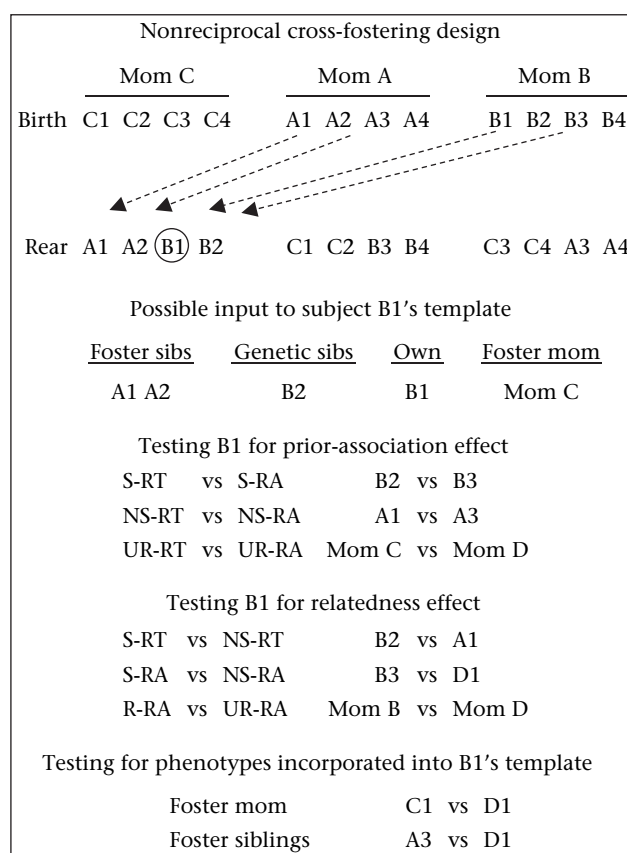

Figure 3. A schematic drawing of the nonreciprocal cross-fostering design in which two (or more) individuals from one litter and two (or more) from another litter are exchanged reciprocally between litters. Dashed lines identify only some of the individuals that are exchanged between litters. As explained in the text, this design can be used to determine whether a rearing mother's phenotype is incorporated into the templates of the young she rears. The litters from which some individuals (e.g. Mom D, D1) derive are not shown. Abbreviations as in Fig. 1.

and unfamiliar nonkin (e.g. test B1 with A3 and D1). This nonreciprocal design can also be used to answer some of the questions addressed by multiple- and single-transfer designs, although the nonreciprocal design may be impractical if the number of potential foster parents (litters) is limited or if births are not synchronous among litters.

The production component of the kin recognition process refers to the phenotypic cues that render individuals (or sets them) distinct from others (Beecher 1988; Reeve 1989), and the nonreciprocal transfer design (Fig. 3) is especially useful for investigating the temporal development of the production component involved in PA and PM. In bank swallows, for example, the 'signature calls' that make chicks individually distinctive do not develop until nestlings are 15–17 days of age (Beecher et al. 1981), which means that chicks' vocal cues could not become part of a kin template until young are at least 2 weeks old. The flank gland odours that golden hamsters can use to recognize their kin are not produced by juveniles until about 30 days of age (Algard et al. 1966), suggesting that, during early development, template formation in young could reliably incorporate their mother's cues but not those of their siblings (Heth et al. 1998; Mateo & Johnston 2000; note that young hamsters may produce other kin-distinct cues). By using a nonreciprocal transfer design (Fig. 3) and switching young at different ages, one could determine if and when agemate or parental cues are assimilated into recognition templates. It could also be used to determine whether the lack of preferential investment by cuckolded males in their own genetic offspring is due to the absence of recognition cues (chicks 'concealing' their identity) or to the absence of males' offspring-discrimination abilities (e.g. Hatchwell et al. 2001; see also Beecher 1988; Kempenaers & Sheldon 1996). In a functional framework, if males mate polygynously it may be especially important for developing young to assimilate into their recognition templates their siblings' cues because their mother's cues alone would not allow recognition of paternal kin such as paternal half-siblings.

Other Issues Concerning Cross-fostering and Recognition Studies

Sex effects

Whether one uses a multiple- or single-transfer design to study kin recognition, the results of subsequent discrimination tests may depend on the sex of the cross-fostered individual. In the avian sexual imprinting literature, for example, some early work suggested that fostered males, but not females, imprint on their foster parents, although subsequent work suggests that this putative sex difference may not exist (reviewed in ten Cate & Vos 1999). However, adult male domestic sheep (Clun Forest, Welsh Mountain, Dalesbred) that had been reared by foster-mother goats (Saänen) preferred females of their foster species as mates, whereas fostered females preferred males of their genetic species (Kendrick et al. 2001). Finally, paired-encounter tests with cross-fostered Belding's ground squirrels showed that females discriminated behaviourally between their unfamiliar 'sisters' and 'nonsisters' whereas males did not treat their unfamiliar 'brothers' and 'nonbrothers' differentially (Holmes & Sherman 1982; but see Mateo 2002). If there are distinct adult sex differences in mate choice or nepotism, investigators should design cross-fostering studies to ensure that effects on both sexes can be assessed.

The meaning of 'template'

If 'template' is a useful heuristic for conceptualizing recognition abilities, one can ask whether templates consist of exemplars (e.g. specific memories of individuals' phenotypes) or prototypes (a single amalgam or gestalt of several individuals' phenotypes). In many species, individual relatives, such as sibling A or sibling B, can be discriminated (e.g. Rendall et al. 1996; Todrank et al. 1998), indicating that some specific representation of these individuals may comprise part of an animal's template. But it is unclear whether these separate representations would be used when unfamiliar kin, such as a paternal half-sibling, are encountered (necessitating multiple comparisons between each representation and the stranger's phenotype) or whether a gestalt template would be used (requiring just one global comparison). Functionally, the outcome would be the same regardless of whether exemplars or prototypes are used, particularly if the referents in a template are weighted according to their relatedness to the animal (e.g. full siblings' traits are emphasized more than half-siblings' traits in either template scenario). Given the ability of some animals to both recognize individual close kin and to 'recognize' unfamiliar nonkin through PM, templates may be both exemplar and prototype based, depending on the recognition context (see also Breed & Bennett 1987). Whatever the case, cross-fostering offers an experimental means to investigate when and how templates develop, and how different potential referents might be weighted in the template.

Interpreting negative results

Kin recognition is an unobservable internal process of assessing genetic relatedness that can only be inferred on the basis of discrimination, the observable differential treatment of conspecifics based on cues correlating with relatedness. Thus, whether one uses preference tasks, rates of social interactions or mate choice behaviours as an index of recognition, it is important to note that a lack of differential treatment cannot be interpreted as a lack of recognition ability (Byers & Bekoff 1986; Waldman et al. 1988; Gamboa et al. 1991). For example, using a single-transfer cross-fostering design, Penn & Potts (1998) did not find evidence that female mice use their own MHC-based cues to avoid mating with unfamiliar kin. None the less, it is possible that mice have the ability to use self-matching but express it in nepotistic contexts, such as communal nesting decisions (Manning et al. 1992), rather than in mate choice contexts. Multiple-transfer cross-fostering studies with several species of voles indicate that social interactions are influenced by familiarity but not relatedness, despite the wide range of social systems among *Microtus* (Gavish et al. 1984; Boyd & Blaustein 1985; Ferkin & Rutka 1990; Berger et al. 1997; Paz y Miño & Tang-Martinez 1999; Fadao et al. 2000). However,

nondyadic tests, such as odour perception tasks, could reveal phenotype-matching abilities in some vole species, even if this ability to discriminate among unfamiliar conspecifics is not expressed in all contexts (e.g. Ferkin & Rutka 1990; Mateo 2002).

Areas for future consideration

Our cross-fostering discussion has focused on the behavioural development of young, yet the designs we detailed can be used to study the development of parents' abilities to recognize their offspring (Michener 1974; Beecher et al. 1981; Holmes & Sherman 1982; Linsenmair 1987). Because the adaptive problems faced by parents and their offspring differ, it would be valuable to determine whether recognition is symmetric or whether only one member of the parent–offspring dyad can recognize its relative (Insley 2001).

Cross-fostering has been widely used with mammals, anurans and invertebrates, but it is an underutilized technique for studying the kin recognition abilities of birds (but see ten Cate & Vos 1999 for its use in studies of imprinting). Although there are many circumstances in which birds could use PM to assess their relatedness to conspecifics (e.g. multiply sired clutches; cooperative breeding among closely related individuals; identification of siblings hatched in different years), few published studies describe individuals being transferred to determine whether birds can use PM to recognize relatives (but see Kempenaers & Sheldon 1996; Hatchwell et al. 2001). In such studies, discrimination tasks should be developed that allow researchers to investigate recognition abilities that may be masked by social contexts such as parental feeding or mate choice decisions.

Finally, when testing fostered animals for recognition abilities, one should consider carefully how to present test stimuli. Using a randomized sequential stimulus presentation, one may better determine an animal's true preference for each. However, younger animals may not continue to respond to stimuli after repeated testing, in which case simultaneous presentations may be recommended (see also Wagner 1998). For all test paradigms, it is critical for observers to be blind to the identity of the stimuli (e.g. whether they are kin or nonkin, familiar or unfamiliar). We hope that our discussion clarifies the issues concerning transfer techniques and that it stimulates the use of cross-fostering designs by researchers broadly interested in the effects of early social experiences on behavioural development.

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