Chapter 22 Scent Marking

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THE SCENT MARKS OF RODENTS have been cast as an olfactory equivalent of the elaborate and colorful train of the peacock (*Pavo cristatus;* Penn and Potts 1998a). This is a helpful analogy, illustrating the importance of scent marking in rodent sexual selection. Just as peahens prefer males with the showiest trains and gain fitness benefits through mating with them (Petrie et al. 1991; Petrie 1994), so female rodents use scent marks of males when choosing mates (as, indeed, do females of many other mammals). However, the analogy tells only part of the story, for scent marking is also inextricably linked with competition over resources and mating opportunities, usually between males. In this sense, scent marking resembles, for example, the roars of red deer stags (*Cervus elaphus;* Clutton-Brock and Albon 1979; Clutton-Brock et al. 1979), on the basis of which potential combatants assess their relative competitive ability and decide whether to challenge an opponent physically. In rodents, as in most mammals, scent marking is a means by which individuals assess the competitive ability of opponents (Gosling 1982, 1990; Gosling and Roberts 2001a). This may occur remotely, before an encounter occurs, or in conjunction with further assessment face to face. While there is variability in, and some debate about, the mechanisms involved, there is little doubt that scent marking is a fundamental component of territorial behavior and of advertising dominance status within social hierarchies.

The benefits of being chosen as a mate or controlling access to mating opportunities account for most, if not all, scent-marking behavior. Evidence from across mammals suggests that scent marking initially evolved as a component of competitive behavior between same-sexed individuals (usually males, although females often scent mark) and that it subsequently became used in mate choice (usually by females; Gosling and Roberts 2001a). The possibility remains, however, that scent marks of males, in some cases, are signals directed specifically to females (Gosling and Roberts 2001a).

In this chapter, I review the wealth of recent rodent studies in light of the view that scent marks are signals of status. Having described the principal glandular sources and behaviors involved, I summarize the evidence that marking is involved in intrasexual competition and mate choice. The evolution of scent marking depends, as in all signals, on the reliability of information that marks contain, and I outline some ways rodents keep signals honest. These ways include major mechanisms by which information carried in scent marks is transmitted to receivers, and key processes (e.g., signal cost, individuality, memorability) that are prerequisites for various mechanistic and functional explanations for scent marking. Finally, I describe some new research directions that may become a focus for the future, including the need for more field studies to validate and test many of the ideas discussed here, which have been largely driven from the laboratory.

Scent Sources and Scent-Marking Behavior

Rodent scent marks emanate from a variety of glandular sources (table 22.1; reviewed in Brown 1985b; Halpin 1985; Macdonald 1985). Urine and anal gland secretion are the

Table 22.1 Distribution of odor sources in major rodent families

Family	OR	HG	MG	EG	DG	VG	PG	CG	AG	PR	UR	PP	VG	Other sources
Aplodontidae	+										+			
Sciuridae	+				+				+	+	+			
Geomyidae	+													
Heteromyidae	+				+	+	+	+			+	+		
Castoridae							+		+		+	+		Castor gland
Pedetidae									+	+				
Cricetidae	+	+	+	+	+	+	+	+	+		+	+	+	Clitoral gland; flank gland; hip gland; neck gland
Spalacidae											+			
Muridae	+	+	+	+		+	+	+	+		+	+	+	Cheek gland
Caviidae									+	+	+			Coccygeal gland; chin gland
Hydrochoeridae									+		+			Morrillo
Dasyproctidae									+		+			
Chinchillidae											+			
Capromyidae									+		+			
Octodontidae											+			
Erethizontidae											+			

SOURCE: Adapted from Brown (1985b), Halpin (1985), and Macdonald (1985).
NOTES: OR = oral glands, lips, saliva; HG = harderian gland; MG = meibomian gland; EG = ear gland; DG = dorsal gland; VG = ventral (or mid-ventral) gland; PG = pedal gland (including plantar glands); CG = caudal gland; AG = anal gland; PR = perineal gland; UR = urine; PP = preputial gland; VG = vaginal secretion.

commonest odor sources. Most species use at least two sources, while others have several. For example, Libyan jirds (*Meriones libycus*) use urine and oral, gular, palmar, plantar, abdominal, preputial, and clitoral gland secretions (Djeridane 2002).

Why do certain species use multiple sources of scent? Differences in number of scent sources at the family level may be partially explained by variation in species-richness, but may also reflect strength of selection on signaling. In *Microtus* species, for example, the number of distinct sources correlates with degree of sociality (Ferkin 2001). Information available in different glands may be additive, though there may also be some redundancy. Lai et al. (1996) compared responses of Djungarian hamsters (*Phodopus campbelli*) to same- or opposite-sex scents. Females investigated male urine and mid-ventral gland secretion (MVGS) more than female scents from the same sources, suggesting redundancy in urine and MVGS at least in terms of gender recognition (similar patterns with different odor sources occur in meadow voles, *Microtus pennsylvanicus;* Ferkin and Johnstone 1995). In contrast, male hamsters respond to different female odors depending on their reproductive status (Lai et al. 1996). Mouth and urine odors were only attractive during postpartum estrus, and attractiveness of vaginal odor peaked at estrus, while MVGS was most attractive immediately before parturition. These temporal differences indicate additive information in different odors and suggest that together they provide a more precise record of individual condition than does one source alone.

Scent deposition takes a variety of forms and specialized behavior patterns. Urination and anal dragging are the most common application behaviors. That urine marking is communicatory, not simply eliminatory, is illustrated by behavioral differences associated with social rank. Dominant laboratory mice (*Mus musculus*) deposit urine in numerous small spots, subordinates typically creating large pools (Desjardins et al. 1973; Bishop and Chevins 1987). South American maras (e.g., *Dolichotis patagonum*) forcibly project urine sprays toward conspecifics (Taber and Macdonald 1984). Kangaroo rats (*Dipodomys* spp.) deposit dorsal gland secretion by rubbing themselves in sandbathing sites (Randall 1981, 1987b). Beavers (*Castor* spp.) actually create marking sites, earth mounds (Aleksiuk 1968), on which they place castoreum, a mixture of castor and anal gland secretion and urine. Artificially constructed mounds elicit normal behavioral responses when presented with castoreum, but not without it (Schulte 1998; Rosell et al. 2000).

Almost all scent-marking studies document variation according to at least one, and often to all, of the following factors: age, sex, physical condition, and season. As a generalization, scent marking is more frequent when animals are adult, male, and dominant or territorial, especially during breeding. This variation is not particular to rodents; the same applies to most mammals (Gosling and Roberts 2001a) and some other taxa (e.g., Moore et al. 1995). Careful documentation of these qualitative differences is the key to understanding the evolution and function of scent marking.

Function of Scent Marking

Functional paradigms

Historically, a variety of functional explanations have been proposed for scent marking (reviewed by Gosling 1982, 1990). Examples include the idea that marks aid in self-orientation within territories (Kleiman 1966; Walther 1978) or in monitoring resource use by providing information about previous visits to feeding sites (Henry 1976; Harrington 1981; Rozenfeld et al. 1994). Such explanations largely depend on levels of exclusive use of space or resources that are unwarranted given actual observations, and cannot readily account for qualitative differences in marking associated with sex, age, status, and season. Similarly, marking behavior in monogamous species has been interpreted as functioning in pairbond maintenance (e.g., Peters and Mech 1975) but can also be explained by general principles that apply equally across mating systems, such as intrasexual competition over mates (Gosling 1982; Roberts and

Dunbar 2000). More promising alternatives included proposals that scent marks deter or intimidate territorial intruders (Hediger 1949; Geist 1965), but these suggestions are not supported by observations, especially since intruders do not usually retreat upon finding a mark.

Gosling instead proposed that scent marking by resource holders provides a means of competitor assessment, signaling fitness costs of trespassing to receivers. These costs are a product of the probability that the signaler will return and of its relative competitive ability (Gosling 1982, 1990; Gosling and Roberts 2001a). On detecting scent marks, receivers have three main options: either withdraw from an area immediately, remain but withdraw on encountering the owner, or remain to further assess the owner, perhaps even deciding to mount an ownership challenge (fig. 22.1). Which option receivers take will be influenced by the potential costs signaled in the mark, the value of the marked resources, the costs of injury, and the scale of assessment error. This may be why responses of receivers to scent marks are so variable. Signalers also benefit because receiver re-
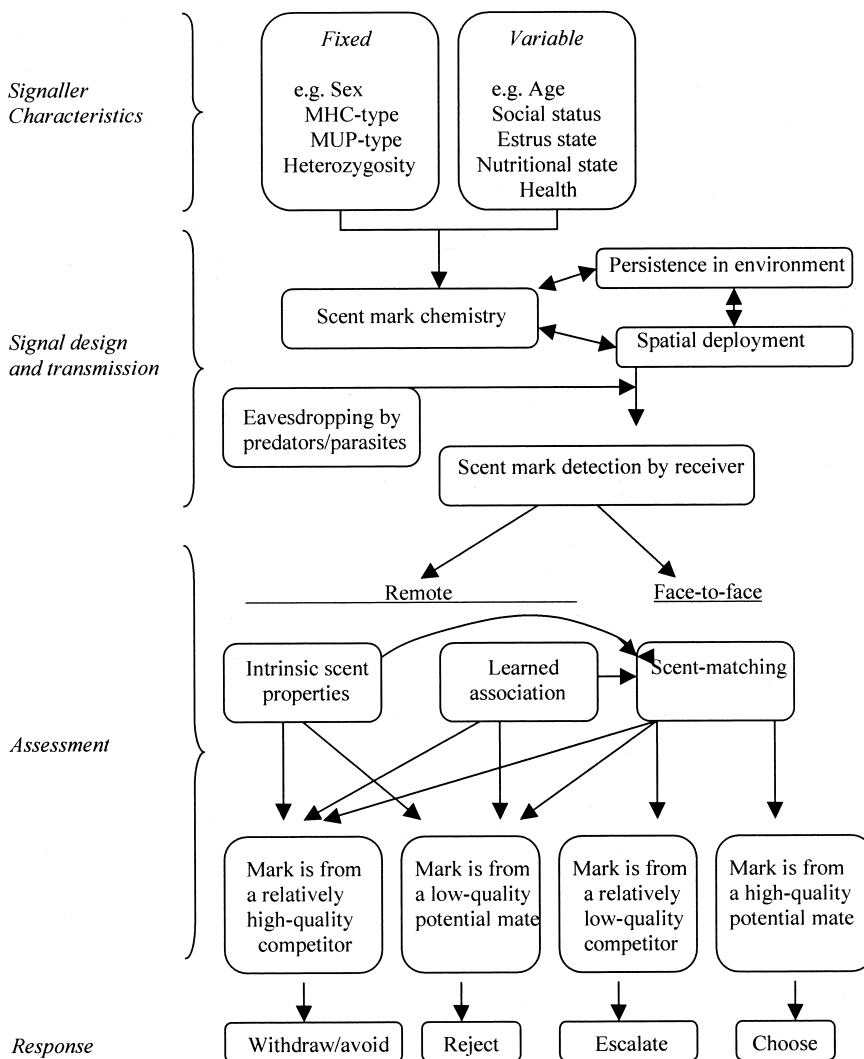
Figure 22.1 Summary of scent marking processes. The assessment and response phases are simplified for illustrative purposes, but in reality responses are complicated by many factors including the value to same-sex receivers of the marked resource, costs of fighting over it and the probability that the receiver will meet the signaller. If receivers are potential mates, responses may vary according to, for example, sexual receptivity, genetic dissimilarity, and the suitability of the signaller in comparison to others already encountered.

sponses reduce the number and intensity of potentially costly fights to which they are exposed. The formulation of scent marks as status signals builds on conventional competitor assessment and game theory (e.g., Parker 1974; Maynard Smith 1982, 1996), providing a clearly stated, unifying theoretical paradigm for understanding scent marking alongside well-developed models of visual and acoustic signaling.

This paradigm can easily be extended to incorporate signal reception by mates (Gosling and Roberts 2001a). Should females be receivers, the nature of the decisions involved may differ, but the reliability or "honesty" (Zahavi 1975) of the signal will still apply. Crucial to the idea that scent marking is an honest signal are findings that male scent-marking rates, associated gland sizes (Horne and Ylönen 1998), and attractiveness to females (Drickamer 1992a) are heritable. Signal costs are discussed more fully later in this chapter.

Despite the growing consensus that scent marks are status signals, the scent-marking literature remains replete with a variety of functional interpretations and subject to a fair amount of debate. Failure to take full account of the complexities of scent-marking behavior can lead to premature rejection of status signaling as a function of marking (see Gosling and Roberts 2001b). Confusion also exists between function and mechanism. For example, Sun and Müller-Schwarze (1998b) conclude that scent matching is the function of marking behavior in beavers, rather than a mechanism mediating a territorial defense function. Scent marks are also sometimes asserted to function as signals of individual identity (e.g., Wolff et al. 2002). Individually specific scent properties may be a necessary precondition for various functional explanations, but they do not provide an explanation of the fitness benefits gained from scent marking. Similarly, the idea that the function of scent marking is self-advertisement (that is, purely communicating presence in an area; e.g., Thomas and Kaczmarek 2002; Thomas and Wolff 2002) does not explain qualitative variation in marking behavior and responses to marks among individuals of different status.

Self-advertisement and other characteristics of marking behavior (e.g., scent individuality, countermarking, overmarking, scent masking) are integral properties or processes involved in status advertisement but are not functions in themselves. Nonetheless, there has been gradual movement over the past two decades toward a consensus view that scent marks are status signals allowing competitor and mate assessment. The following sections outline pertinent evidence arising from rodent studies.

Scent marks and male intrasexual competition

There is overwhelming evidence that scent marking is involved in intrasexual competition among males (reviews in Ralls 1971; Johnson 1973; Brown and Macdonald 1985; Gosling 1990; Gosling and Roberts 2001a). Evidence pointing to this association includes (1) correlations between social status and both scent marking and responses to marks, (2) links between frequency of scent marking and strength of intrasexual competition, (3) nonrandom deposition of scent marks within territories, (4) correlations between qualitative differences in scent chemical composition and social status, and (5) demonstration that marking and glandular development are often androgen dependent.

1. Individuals of elevated social status (i.e., dominant or territorial males) typically mark at higher frequencies than low-status males. For example, this occurs in laboratory mice (Bishop and Chevins 1987; Gosling et al. 2000), house mice (*Mus domesticus;* Hurst 1990a, 1990c), capybaras (*Hydrochaeris hydrochaeris;* Herrera and Macdonald 1994) and bank voles (*Clethrionomys glareolus;* Rozenfeld et al. 1987). Dominant male mice are quick to overmark the marks of other males (Hurst 1990b). Rates of scent marking by young mice are the best predictor of dominance in later life (Collins et al. 1997).

Correlations are also evident between social status and responses to scent marks. Males of several species of rodents avoid scent-marked substrates, especially when they are of low competitive ability (Gosling et al. 1996a, 1996b; Lai and Johnston 2002; Luque-Larena et al. 2002c) or the scent is from dominant males (Summerlin and Wolfe 1972; Jones and Nowell 1989; Hurst et al. 1994). Males avoid prolonged fights with males whose scent suggests they are territory owners (Gosling and McKay 1990; Hurst et al. 1994; Luque-Larena et al. 2001). In addition, male mice that were defeated in interactions display prolonged inhibition of urine marking compared with nondefeated controls (Lumley et al. 1999).

2. Scent-marking effort is associated with levels of intrasexual competition. Simulated territorial intrusions by males stimulate increased marking in male blind mole-rats (*Spalax ehrenbergi;* Zuri et al. 1997), beavers (Rosell et al. 2000; Rosell and Bjorkoyli 2002), and alpine marmots (*Marmota marmota;* Bel et al. 1995). Dominant male mice increase marking frequency as subadults within their territory mature (Hurst 1990b).

Increased marking frequencies also influence investment in the glandular structures that produce secretions. Among dominant male mice, those smaller than their subordinates scent mark at higher frequencies and develop absolutely larger preputial glands than relatively large dominants, in-

dicating they have to work harder to establish and maintain social status due to their relative size disadvantage (Gosling et al. 2000). Similarly, gland weights of male house mice housed with another male for 2 weeks grow to a size almost double that of isolated males (Bronson and Marsden 1973). Males housed adjacent to intact mice develop larger glands than controls housed next to castrates, whereas glands of males housed next to females become smaller (Hayashi 1986). Regular exposure to unfamiliar scent also increases gland size in receivers (Hayashi 1990). Unaggressive strains of mice have smaller glands than aggressive strains (Yamashita et al. 1989). In bank voles, dominance is correlated with preputial gland size (Gustafsson et al. 1980), while in coypus (*Myocastor coypus*) anal gland sizes are predicted by numbers of male but not female recruits into a wild population (Gosling and Wright 1994).

In addition to absolute size, differences in glandular structure exist between individuals of different status. Preputial glands of dominant male mice were well-developed with acini at different stages of maturation, many cytoplasmic organelles, and healthy oval-shaped nuclei. In contrast, subordinates had less developed glands with fewer cellular organelles and shrunken, lobulated nuclei (Brain et al. 1983).

3. Territorial males deposit marks where they are more likely to intercept intruders. Spatial clustering of marks toward territorial boundaries is often found in mammals (Gosling 1981; Gosling and Roberts 2001a), including rodents (e.g., Bel et al. 1995). In blind mole-rats, experimentally manipulating intrusion pressure induces spatial shifts in marking effort (Zuri et al. 1997). In beavers, marks are clustered toward territorial boundaries and upstream of lodges, reflecting the direction of emigration from natal territories (Rosell et al. 1998; Schulte 1998). In addition, more mounds are formed along densely populated large rivers than small ones, probably reflecting intrusion pressure from potential immigrants (Ulevicius and Balciauskas 2000). Individuals also mark along paths and at burrow entrances (e.g., Banks and Banks 1979; Ferron and Ouellet 1989b; Rozenfeld et al. 1994; Blumstein and Henderson 1996; Brady and Armitage 1999). However, an absence of well-defined spatial patterns is not necessarily evidence against a territorial role for marking, because factors that are spatially heterogeneous (e.g., resources, intrusion pressure) may also influence marking economics (Roberts 1997; Roberts and Lowen 1997; Gosling and Roberts 2001b).

4. Chemical differences exist in the scent marks of males of different status. The best evidence comes from house mice, where chromatographic comparisons reveal quantitative differences in sixteen urinary compounds between dominant and subordinate males (Harvey et al. 1989). These characteristic differences can arise within 7 days of status establishment: concentrations of urinary dihydrofurans, ketones, and acetates decrease in subordinate urine, while 2-(*sec*-butyl)-4,5-dihydrothiazole and two sesquiterpenic compounds, alpha- and ß-farnesene, increase in dominant urine. The farnesenes are the two most prominent constituents of preputial gland secretions (Novotny et al. 1990), which as previously described are closely linked to dominant behavior.

5. If scent marking is involved in intrasexual competition between males, it would be surprising if the secretion-producing glands were not androgen dependent. Evidence for androgen dependence is suggested by correlations between mass of testes and scent glands, such as the morrillo of adult capybaras (Herrera 1992). Similarly, endogenous testosterone levels of adult male gerbils are correlated with ventral gland size and frequency of scent marking (Clark et al. 1990, 1992b).

Androgen dependence has been conclusively demonstrated by castration and androgen restoration. In house mice, prolonged investigation of an area is inhibited by scent marks. Castration of scent markers eliminates this effect, while testosterone treatment reestablishes it (Jones and Nowell 1973; Sawyer 1980). These behavioral effects are probably due to changes in the chemical constituents of urine in relation to testosterone levels (Novotny et al. 1984; Harvey et al. 1989). The four principal compounds of dominant males (farnesenes, dihydrothiazole and dehydro-*exo*-brevicomin) are not present in urine of castrates but are restored by testosterone treatment (Harvey et al. 1989). In Mongolian gerbils (*Meriones unguiculatus*), atrophy of preputial and abdominal sebaceous glandular tissues is induced by castration and restored by testosterone (although there is no effect on oral, gular, palmar, and plantar glands; Djeridane 2002). Similar effects on inhibition of marking behavior are known in Mongolian gerbils (Arkin et al. 2003), Long-Evans rats (*Rattus norvegicus*: Matochik and Barfield 1991), European ground squirrels (*Spermophilus citellus;* Millesi et al. 2002), and tree shrews (*Tupaia belangeri;* Holst and Eichmann 1998).

Scent marks and female choice

Male scent marks influence females in two main ways. First, chemical constituents of marks elicit physiological, or priming, responses in females (Driekamer chap. 9 this volume). Second, females use information in marks during mate choice. As the higher-investing sex, females should choose

males in relation to their mate quality (Bateman 1948; Trivers 1972), and the inherent costs involved ensure that scent marking is a reliable indicator of male condition (Gosling and Roberts 2001a).

Females respond selectively to male odors and positively to males of high status or quality. For example, the scent of well-nourished males attracts more interest than the scent of poorly nourished males (Ferkin et al. 1997). Females prefer the odor of dominant males to that of subordinate ones in laboratory mice (Parmigiani et al. 1982), rats (Carr et al. 1982), bank voles (Hoffmeyer 1982; Horne and Ylönen 1996; Kruczek and Pochron 1997), and water voles (*Arvicola terrestris;* Evsikov et al. 1995). In laboratory mice, this preference was greater when dominants regularly encountered new rivals than when dominants were exposed to a single intact subordinate, while the latter, in turn, were more attractive than males housed with castrated subordinates (Hayashi 1990; see also Scott and Pfaff 1970; Hayashi and Kimura 1978). Preputial gland removal nullifies female preferences for dominants (Hayashi 1990). Neurons in the olfactory bulb, preoptic area, and lateral hypothalamus respond differentially to intact and castrate odors (Scott and Pfaff 1970). The chemical basis for these preferences is also known: volatile chemicals associated with dominance (as described earlier in this chapter) are attractive to females (Jemiolo et al. 1985, 1989).

Females prefer males whose pattern of odor deposition indicates high quality or resource-holding potential (RHP), such as males whose territories contain only the owner's marks (Rich and Hurst 1998), and those that countermark intruder scent (Johnston et al. 1997a; Rich and Hurst 1999). If females prefer high-status males, and these males invest more in marking, we would also expect females to use marking frequency as an indicator of male quality. In gerbils, intrauterine position correlates with circulatory testosterone levels (Clark et al. 1992b), larger scent-marking glands, and higher marking rates, which females also prefer (Clark et al. 1992a). Marking rate also predicts female preference in laboratory and house mice (Roberts and Gosling 2003; Zala et al. 2004).

Studies finding no indication of female preference based on marking frequency (e.g., Thomas 2002; Mech et al. 2003) emphasize that frequency is a proximate indicator of quality that is modulated by social conditions and that correlates with other aspects of marking behavior, including scent chemistry. This may explain why artificially increasing apparent marking rates by collecting and presenting to females many scent marks of a particular male may not successfully enhance his attractiveness (e.g., Thomas 2002). Greater success in such efforts may be achieved by altering marking behavior and scent composition more indirectly,

and realistically, through manipulation of social environment or status. For example, in wild-derived house mice (Zala et al. 2004) and in harvest mice (*Mus minutus;* Roberts and Gosling 2004), exposure of males to female or male odor, respectively, increases both male marking rate and scent attractiveness.

Female preferences for the odor of familiar males may also be linked to RHP, since the prevalence of a male's marks reflect dominance or territorial residency (Roberts and Gosling 2004). Preferences for familiar odors are known in prairie voles (*Microtus ochrogaster;* Newman and Halpin 1988), house mice (Heise and Hurst 1994), hamsters (*Mesocricetus auratus;* Lis et al. 1990; Tang-Martinez et al. 1993), kangaroo rats (*Dipodomys merriami;* Randall 1991b), and harvest mice, in which familiarity also reduces male-directed aggression (Roberts and Gosling 2004).

Other studies are consistent with the idea that females prefer males to have costly sexual displays because they indicate health and lower parasite loads (Hamilton and Zuk 1982; Penn and Potts 1998a). Female house mice distinguish odors of parasitized and unparasitized males (Kavaliers and Colwell 1992, 1995a; Kavaliers et al. 2003), and infection reduces odor attractiveness (Penn et al. 1998; Klein et al. 1999; Willis and Poulin 2000). Discrimination of healthy mates is improved when male marking rates are artificially increased (Zala et al. 2004).

Preferences are also mediated by genetic differences at the major histocompatibility complex (MHC), which codes for proteins involved in immune response and thus pathogenic resistance (chap. 5 in this volume). MHC-disassortative mating preferences (Yamazaki et al. 1976, 1979; Potts et al. 1991) benefit females because they increase offspring heterozygosity (e.g., Penn 2002). However, expression of MHC preferences is modulated by, and may trade off against, preferences for other male qualities. In congenic mouse strains, MHC haplotypes are associated with differential investment in scent marking, influencing female preferences more than genetic complementarity under defined circumstances. The interaction between these two qualities suggests a mechanism for maintaining hypervariability in both (Roberts and Gosling 2003).

Scent marking by females

Marking among females has received less research attention than in males, largely because females mark less often and less intensively (e.g., Johnson 1975; Holst and Eichmann 1998; Wolff et al. 2002). However, there is evidence that female marking is associated with intrasexual competition and enhancing opportunities for mating with high-quality mates. Reproductively active female house mice scent mark

at higher frequencies than nonbreeders and preferentially countermark the marks of breeding females (Hurst 1990c). Female hamsters increase scent marking in response to female more than to male scent (Johnston 1977). Rates of aggression and scent marking are typically low in female bank voles housed together, but rise around parturition, when unfamiliar female odors trigger increased aggressiveness and scent marking (Rozenfeld and Denoel 1994). Common vole (*Microtus arvalis*) mothers mark more intensively than daughters, suggesting a link between frequency and status (Heise and Rozenfeld 1999). Female Syrian hamsters are more aggressive in the presence of their own odor than in a blank arena (Fischer and McQuiston 1991) and scent marking appears to be influential in determining low-range overlap in female bank voles (Ziak and Kocian 1996).

Female marking may also be directed toward males rather than female competitors. Female house mice mark at higher rates in the presence of intact males than castrates (Maruniak et al. 1975). When female hamsters mark in the presence of males (Johnston 1977), marks stimulate male aggression and could increase chances of mating with high-quality males (Fischer and Brown 1993). Increased marking frequency during estrus in Long-Evans rats is consistent with advertisement of reproductive status to males (Matochik et al. 1992).

Scent-Marking Mechanisms and Processes

Scent marks are signals that are usually transmitted in the absence of receivers, perhaps without targeting a particular receiver, and usually detected a relatively long time afterward, in the signaler's absence (Gosling 1982). In this regard, scent marking is unique among social signals. How then do receivers react to scent marks that they encounter? Reactions are expected to be highly variable between individuals. Assuming that the receiver is a competitor, responses will be influenced by factors that include the relative competitive abilities of receiver and signaler, the value to each of the marked resource, the costs of fighting over it, and the probability that the two will meet (Gosling and Roberts 2001a). If receivers are potential mates, responses could be influenced by condition-dependent cues of quality (which are likely to be the same that signal RHP to competitors), female receptivity, genetic dissimilarity, and the suitability of the signaler in comparison to others already encountered.

Three main mechanisms that account for how receivers use information available in scent marks have been proposed (Gosling 1990; Gosling and Roberts 2001a; see fig. 22.1). First, intrinsic properties of the marks may reveal information about the signaler. Second, receivers may have learned and remembered the signaler's identity from past encounters, whose outcome influences current behavior. Third, receivers may memorize the odor of the mark, forming a template (Sherman et al. 1997) with which to compare odors of individuals they meet subsequently, with a match between template and odor implying that the individual is the signaler. The first two mechanisms do not require the receiver to meet the signaler, while in the latter case the response is delayed until after an encounter (fig. 22.1).

These mechanisms are nonexclusive and unlikely to be species specific. Thus receivers may have previous experience of the signaler, but can update this information (template updating; Sherman et al. 1997) using current scent properties and ultimately confirm assessment through face-to-face matching, although not all options will necessarily always be used. For example, matching may become more necessary at high population densities, because of increased fighting costs, potential for mistaken identity, and higher turnover of dominant or territorial males. In contrast, potential for learned associations between marks and signaler quality is higher in temporally stable networks. One consequence of learned associations occurs between established territorial neighbors, in which familiarity reduces signaling costs along shared boundaries (the "dear enemy phenomenon"; e.g., Rosell and Bjorkoyli 2002).

Of these mechanisms, scent matching is the most accurate because it potentially takes account of all available information. Early evidence for scent matching comes from a study in which smearing urine from an unknown male mouse onto one member of a male pair resulted in increased aggression within the pair, whereas aggression was reduced if the odor came from a familiar individual (Mackintosh and Goddard 1966). Scent matching has been experimentally demonstrated; intruding males fight less with males whose odor matches the substrate odor (simulated territory owners) than when the substrate is marked by a third male (fig. 22.2; Gosling and McKay 1990). Similarly, snow vole males spend less time exploring scent-marked areas of odor matching than nonmatching males (Luque-Larena et al. 2001). Scent matching also occurs in mate choice contexts (Steel 1984).

Whichever mechanism is prevalent in a particular case, selection should act on signal design to optimize reception, accuracy, and reliability. An excellent review of the design of chemical signals, including scent marks, is provided by Alberts (1992), while Gosling and Roberts (2001a) have reviewed behavioral adaptations to increase signal efficacy across mammals. In rodents, much recent research has focused on a number of key processes that are central to our understanding of scent-marking mechanism and function, and some of these are outlined in the following sections.
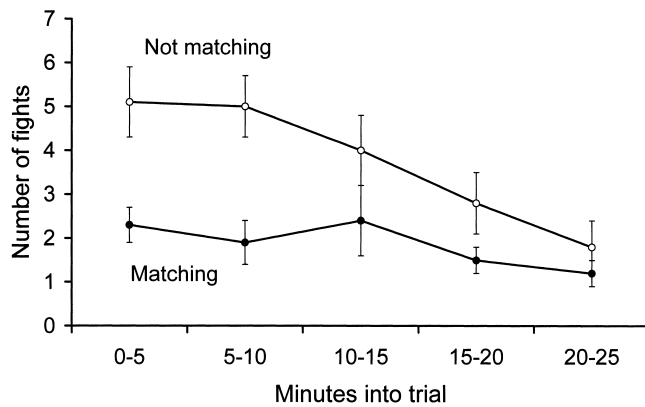
Figure 22.2 Experimental evidence for competitor assessment by scent matching. The mean number of fights (± standard error) in successive 5-min periods of trials where the scent marks on the substrate of the experimental arena either match or do not match the odor of the resident. Twenty-one male mice were tested in both conditions. Fewer fights were recorded when the scents matched. Redrawn from Gosling and McKay 1990.

Signal cost

As in all animal signals, scent marking should be costly if it is used in assessment of quality; otherwise it would be un-reliable and susceptible to cheats (Zahavi 1975). Although marking can account for significant proportions of an ani-mal's time budget, only recently has there been any quan-tification of the energetic costs involved. In rodents, major urinary proteins (MUPs) are synthesised in the liver and ex-creted in urine, their sole apparent function being in chemi-cal signaling (Nevison et al. 2003). Average urinary pro-tein concentrations in house mice are 30 mg ml^{-1}, almost all of which are MUPs (Beynon et al. 2001). In terms of pro-tein turnover, house mice synthesize almost their entire liver weight every 24 hours (h), a substantial energetic cost (Bey-non et al. 2001). Consistent with gender differences in marking behavior, MUP expression is androgen dependent (Knopf et al. 1983). Two studies have estimated MUP con-centration in urine, finding it to be two to three (Beynon et al. 2001) or even five to twenty (Flower 1996) times higher in males than females.

In view of these levels of protein synthesis and secretion, individual variation in scent-marking investment could carry significant metabolic costs. Indeed, in male mice, scent-marking rates are inversely correlated with weight gain in young mice (fig. 22.3; Gosling et al. 2000). In males housed in pairs, dominants that are smaller than their subordinates mark at higher rates than relatively large dominants, grow more slowly, and are consequently more susceptible to dominance reversals (fig. 22.3). Reduced dominance tenure could have critical fitness costs in short-lived species. These results raise the possibility that different investment in scent marking represents alternative mating strategies, where
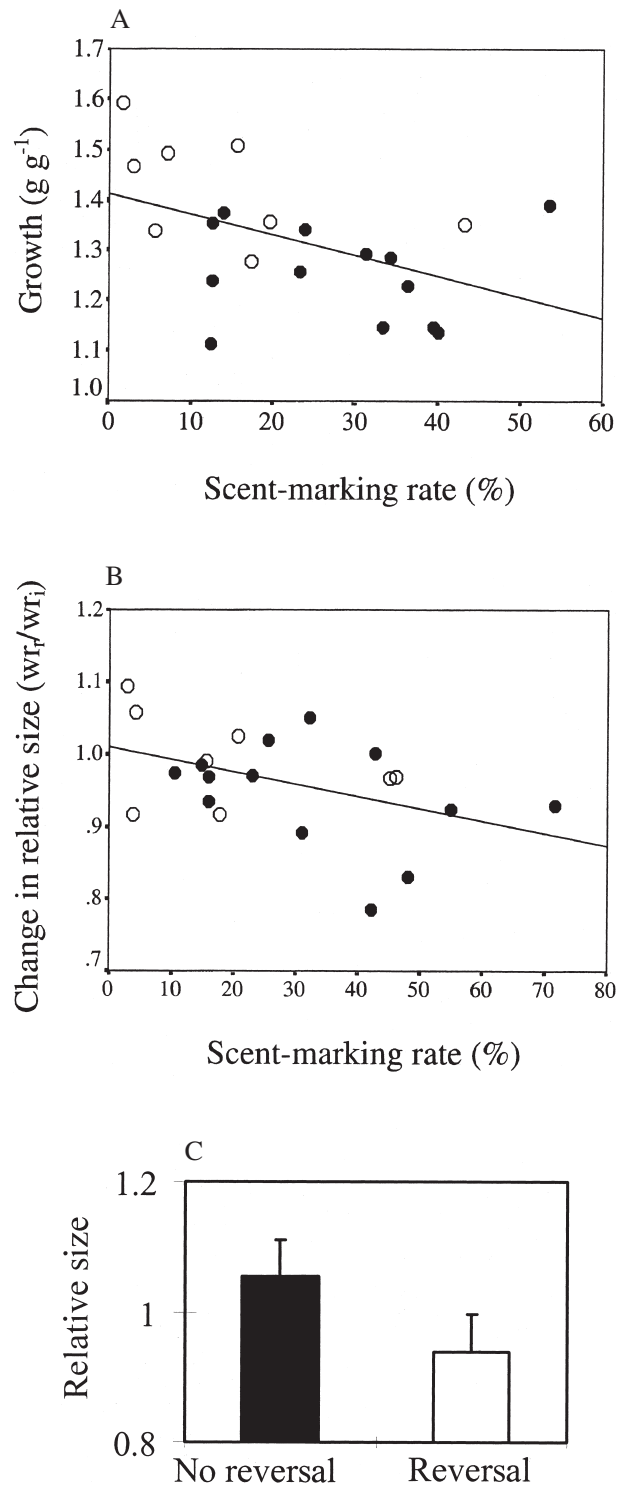
Figure 22.3 Life history costs of investment in scent marking by male mice. (A) In dominant males, mean growth rates between the ages of 9 and 25 weeks are inversely correlated with scent-marking rate ($P = 0.011$). Males were housed with another, subordinate male; closed circles denote the dominants are smaller than their subordinate, open circles denote larger dominants. (B) Dominant males that scent mark at higher rates become progressively smaller compared to their subordinate ($P = 0.038$). (C) Initially dominant males that incurred a dominance reversal were smaller, at the point of reversal, than males that maintained status throughout the experiment ($P = 0.037$). From Gosling et al. (2000).

low-marking, larger males adopt sneak-breeding or waiting strategies (Gosling et al. 2000). Gosling et al.'s study used an outbred laboratory strain (TO) with MUP concentrations of 10−11 mg ml⁻¹ (Nevison et al. 2000), but these energetic costs could be even more significant in wild mice, where MUP concentrations are three times higher (Beynon et al. 2001).

Individual recognition

Individual differences in rodent odors appear to be universal (reviews in Halpin 1986 and Voznessenskaya et al. 1992). Differences are documented from Norway rats (Carr et al. 1970a), laboratory mice (Bowers and Alexander 1967), Mongolian gerbils (Dagg and Windsor 1971; Halpin 1976), chipmunks (*Tamias striatus;* Keevin et al. 1981), prairie voles (Newman and Halpin 1988), cavies (*Cavia aperea;* Martin and Beauchamp 1982), the tuco-tuco (*Ctenomys talarum;* Zenuto and Fanjul 2002), Damaraland mole-rats (*Cryptomys damarensis;* Jacobs and Kuiper 2000), red squirrels (*Tamiasciurus hudsonicus;* Vaché et al. 2001), and golden hamsters (Johnston and Rasmussen 1983; Tang-Martinez et al. 1993). Individual odors may also be recognized across species (e.g., Beauchamp et al. 1985; Johnston and Robinson 1993; Todrank and Heth 1996).

In hamsters, Johnston and Bullock (2001) demonstrated across-odor habituation to different odors from the same individual, indicating that scent from multiple sources potentially reveals individual identity. The vomeronasal organ (VNO) apparently aids discrimination, although VNO removal eliminates this ability only in males and only for certain odor sources (Johnston and Peng 2000).

As odors are influenced by environmental factors such as diet (Ferkin et al. 1997) and stress (Carr et al. 1970b; Kavaliers and Ossenkopp 2001; Marchlewska-Koj et al. 2003), the individual signal component must be discriminable over time. This is assured if odors are at least partially genetically determined. Evidence for a genetic component comes from observations that odor chemical profiles are more similar within closely related species (Heth and Todrank 2000; Heth et al. 2002) and among closely related individual beavers (Sun and Müller-Schwarze 1998a).

Two genetic regions are principal candidates for the basis of individual odors, owing to their polymorphic nature and expression in scent marks. The first is the MHC, known in mice as H-2. Mice and rats discriminate between individuals differing only at MHC (Yamazaki et al. 1979; Brown et al. 1987), even between mice carrying single MHC gene mutations (Yamazaki et al. 1990, 1991; Bard et al. 2000). Discrimination is mediated by varying proportions of volatile carboxylic acids in urine (Singer et al. 1997) and

influences preferences for mates (e.g., Yamazaki et al. 1976; Potts et al. 1991; Roberts and Gosling 2003) and nestmates (Manning et al. 1992).

The second region contributing to individuality is the polymorphic multigene family coding for MUPs (Beynon and Hurst 2003). While MUPs are known to extend the active life of scent marks (Hurst et al. 1998), recent evidence suggest they also have a more fundamental role. Males respond differently to the odor of brothers with different MUP expression but not to those of the same MUP type (Hurst et al. 2001). Countermarking responses depend on having direct contact with urine, suggesting that these involatile signal components are themselves important in individual recognition (Humphries et al. 1999; Nevison et al. 2003). Whether and how MHC and MUP genetic components interact in forming unique odor signatures remain to be addressed.

Memory

Like the ability to recognize individuals, the ability to remember scent mark properties is a key requirement for adaptive responses. That animals remember marks is implicit in many studies investigating marking behavior where responses are linked to previous experience. One common example is where female preference tests between males follow exposure to their marks (e.g., Johnston et al. 1997a; Johnston and Bhorade 1998). Preferences based on this information last for 48h in voles (Ferkin et al. 2001). Similarly, avoidance responses of subordinates to odors of dominant male mice suggest memory for odors and signaler's relative quality (Carr et al. 1970b). These kinds of responses can be directly employed to study memory. For example, Lai and Johnston (2002) showed that males could recognize, remember and avoid odor of a male that defeated them in both the short term (30 minutes after fighting) and the long term (1 week later). Other research uses habituation-dishabituation techniques (fig. 22.4). Flank gland odors are remembered for at least 10 days in hamsters (fig. 22.4; Johnston 1993) and up to 4 weeks in guinea pigs (Beauchamp and Wellington 1984). Perhaps most impressive, Belding's ground squirrels (*Spermophilus beldingi*) can remember and discriminate between familiar versus unfamiliar, and kin versus nonkin, odors after over-winter hibernation (Mateo and Johnston 2000).

Interpreting patterns of marks

Animals often scent mark near, or on top of, marks of conspecifics. This is generally termed *countermarking* (overmarking is a form of countermarking in which the second mark is placed directly over the first mark). In particu-

A

B
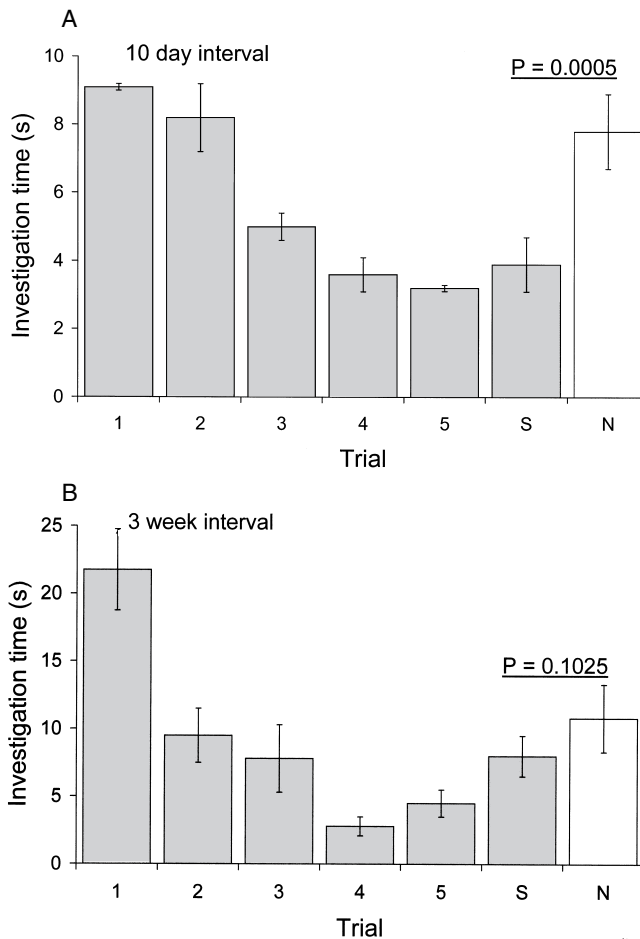
Figure 22.4   Testing memory for individual odors using habituation-discrimination techniques. In five successive trials 15 min apart, the mean number of seconds (± SE) that male hamsters investigated flank gland scent of another male decreases as habituation to the odor occurs. In a sixth trial, responses to scent from the same male (S) and a novel male (N) are compared (discrimination test). (A) Successful discrimination was recorded when the interval between the test trial and the last habituation trial was 10 days, indicating memory for the initially presented odor. (B) There is no difference in time spent investigating each scent when this interval was 3 weeks. Redrawn from Johnston (1993).

lar, resource holders (territorial or dominant males) always countermark the marks of intruders or subordinates, because these may represent a challenge for the resource and/or because it introduces ambiguity into subsequent assessment by receivers (Gosling 1982). Receivers may glean information about relative qualities of local signalers through marking patterns deposited by different individuals at different times. In essence, then, countermarking should be seen as occasional reactive scent marking to maintain the integrity of a marking network previously established and maintained on a more proactive basis.

In an early experiment in this area (reviewed by Johnston 2003), Johnston et al. (1994) showed that, following habituation to simulated hamster overmarks, novel scents are investigated more than top scents in overmarks, sug-

gesting that hamsters had habituated to the top scent. In contrast, the novel and the bottom scents were investigated equally. Johnston et al. suggested that information in the bottom scent might be masked by the top scent. However, since countermarks often do not completely cover existing marks, this experiment was repeated using partially overlapping marks (Johnston et al. 1995) with similar results. This indicates that, rather than being masked, bottom scents simply fail to attract the interest of receivers, perhaps because individuals whose scent is overmarked are apparently relatively unthreatening (see also Woodward et al. 1999).

Further work showed that these effects on receiver behavior were not explained by the area of top and bottom scents available for investigation, since top scents were investigated less even when they occurred in smaller quantities than bottom scents (Wilcox and Johnston 1995). Nor can age differences between the top and bottom marks explain the responses: when Wilcox and Johnston (1995) exposed males to two nonoverlapping scents varying in age by 20–45s (the same interval between artificial deposition of mark and overmark in previous experiments), the males habituated to both scents. This indicates that small differences in age between the two scents are insufficient in themselves to permit discrimination. In mice, however, short intervals between scent depositions (30 s) did not elicit response differences to top- and bottom-scent donors, perhaps because of scent blending while the secretion was still wet (Rich and Hurst 1999).

In view of these findings, what is the mechanism that accounts for the difference in receivers' responses to top and bottom scent? The answer appears to lie in the spatial configuration of marks. Response differences to top-scent donors only occur if a region of overlap exists between the marks. If artificial marks and overmarks are made to appear as though one overlaps the other, even though it doesn't, the response difference is still recorded (Johnston and Bhorade 1998). Similar results have been found in voles (Johnston et al. 1997a, 1997b; Ferkin et al. 1999). These results suggest that rodents are very adept at interpreting these spatial patterns and that the resulting responses may confer fitness benefits.

Mate preference tests show that females prefer top-scent males in hamsters (Johnston et al. 1997a), meadow voles (Johnston et al. 1997b; Ferkin 1999), and house mice (Rich and Hurst 1998, 1999). If countermarking has fitness effects, it should also be modulated by relatedness between potential competitors (and possibly mates). Indeed, in prairie voles, scent marks of siblings received fewer overmarks than marks of unrelated individuals (Kohli and Ferkin 1999). However, despite this evidence, it is likely that patterns of overlapping scent comprise only part of the information that receivers use, and this is illustrated by Leonard

et al.'s (2001) study of gonadectomised meadow voles, in which females take into account both mark position and testosterone titers.

The overmarking studies by Johnston, Ferkin, and colleagues cited previously were designed to interpret the consequences of overmarking to artificially created overmarks and not the frequency or actual placement of overmarks per se. In contrast, a series of studies designed to quantify whether meadow voles and prairie voles overmark found that overmarking occurred less often than expected by chance (e.g., Thomas and Wolff 2002; Mech et al. 2003). These authors concluded that voles attempt to avoid overmarking, perhaps to retain individual identity (see also Thomas and Wolff 2003). Thus even though voles can discriminate top- from bottom-scent donors, and even show a preference for one over the other, overmarking in voles may not be an adaptive or sexually selected trait.

Eavesdropping

Another potential cost of scent marking is that signals may be intercepted by individuals other than the intended receivers, to the signaler's disadvantage. For example, young males may use information in marks to monitor the status of local resource-holding males, with a view to challenging poor males for the resource, or females may use the same information to avoid poor-quality mates (e.g., Rich and Hurst 1999). In addition to conspecific eavesdroppers, signalers could also alert predators to their presence, location, and movements, which could carry particularly high costs. Diurnal avian predators like the kestrel (*Falco tinnunculus;* Viitala et al. 1995), rough-legged buzzard (*Buteo lagopus;* Koivula and Viitala 1999), and great grey shrike (*Lanius excubitor;* Probst et al. 2002) can detect vole scent marks (*Microtus, Clethrionomys*) and focus hunting effort in densely marked areas (see also Koivula and Korpimäki 2001). Detection is mediated by ultraviolet (UV) reflectance of proteins in the marks. Kestrels discriminate between age and sex classes, preferring male field voles (*Microtus agrestis*) over females and juveniles (Koivula, Viitala, and Korpimäki, 1999), apparently using differences in UV reflectance between classes (Koivula, Koskela, and Viitala, 1999a).

Predation rates by terrestrial predators like mustelids may be higher than predation by raptors (Koivula and Korpimäki 2001). Here, prey odor, rather than mark visibility, may be the important cue to prey availability. Preferences of least weasels (*Mustela nivalis nivalis*) for odors of different reproductive categories of bank voles in the laboratory did not reveal the same kind of discrimination as found in raptors, although weasels preferred vole odors over the clean arm of a Y-maze (Ylönen et al. 2003). In the field, however, areas with artificially elevated scent-mark densities were

hunted more intensively and vole survival was lower, suggesting that marking density attracts greater hunting effort (Koivula and Korpimäki 2001).

Males that invest more in scent marking, through high protein concentrations in marks and/or through marking at high rates, may thus be at higher risk than females and low-investing males. Males may therefore be expected to invest less in scent marking in predator-rich areas, even temporarily reducing marking at times of high risk. Evidence for this hypothesis includes the finding that exposure to weasel odor causes reduction in hamster flank gland size, among a number of physiological effects (Zhang et al. 2003). The idea was further tested by Roberts et al. (2001), who used sib-sib comparisons to examine the degree to which male mice of known signaling investment countermarked scent marks of an unfamiliar individual in the presence or absence of predator odor (urine of ferrets, *Mustela putorius furo*). Under simulated predation risk, all males approached the competitor's marks more slowly, although high-frequency markers approached more quickly than low-frequency markers and spent more time in the vicinity of the competitor's marks. Only high-investing males significantly reduced overmarking of the competitor's scent in the presence of predator odor. These results suggest there is a unique danger inherent to scent marking at high frequencies and that high-investing males were prepared to accept increased costs of intrasexual competition to reduce the risk of predation.

In contrast, a recent study of marking by prairie voles and woodland voles (*Microtus pinetorum*) found no evidence of reduced marking in response to odor of minks (*Mustela vison*) and bullsnakes (*Pituophis melanoleucus;* Wolff 2004). Marking rates were tested both in large enclosures and in the laboratory. Some methodological differences exist between the laboratory component of this study and that of Roberts et al. (2001) that could account for the different results. In the latter study, use of sibling comparisons controlled for potentially genetic differences in marking effort (see Collins et al. 1997), the cage environment was relatively complex with reduced visibility (i.e., perceptually more dangerous), and marking was measured in response to competitor scent (counter marking), rather than marking in a blank area. However, more work is clearly needed, both in the laboratory and the field, to determine the potential sensitivity of scent-marking effort to predation risk.

Conclusions and Future Directions

One reason rodents have made such an impact on our understanding of scent marking is because of their amenability to laboratory studies. Recently, however, efforts have been

made to validate some findings in the field, with mixed success (Mahady and Wolff 2002; Wolff 2003c, 2004), although some excellent examples of transferability of results from lab to field exist. Notable among these are observations that MHC-disassortative mating preferences discovered in the laboratory (Yamazaki et al. 1976) influence mating patterns in free-ranging mice (Potts et al. 1991). Thus while controlled conditions in the laboratory offer unique opportunities for research and should remain important in the future, Wolff's call for field-validation of laboratory findings (Wolff 2003c) must be heeded to improve confidence in results obtained from laboratory studies.

Studies of scent-marking behavior in rodents are opening up a range of new research directions. Two notable examples are (1) approaches in neuroscience that are revealing neural pathways involved in scent marking and olfactory perception, and (2) application of our understanding of scent marking in animal welfare and conservation biology.

Increasing research effort focuses on the neural control of marking behavior. A comprehensive review is beyond the scope of this chapter, but some examples are provided here. One focus has been the role of vasopressin (VP) in regulating social behavior in general and scent marking in particular. Microinjection of VP into several areas of the brain stimulates flank-gland marking in Syrian hamsters, while lesions of the same areas inhibit it (Hennessey et al. 1992; Albers and Bamshad 1998). VP-containing neuronal cells and fibers in the neurohypophyseal system and several extrahypothalamic areas are sexually dimorphic and androgen dependent, and control scent marking in a sex-specific manner (Dantzer and Bluthe 1992). Galanin, which antagonizes postsynaptic action of other neurotransmitters, also blocks VP-induced flank marking, suggesting that endogenous galanin may be an inhibitory force in scent-marking behavior (Ferris et al. 1999). Norepinephrine has a similar, dose-dependent effect (Whitman et al. 1992). Other research combining lesion and behavioral studies shows that the parahippocampal region is important in individual odor discrimination (Petrulis et al. 2000), while the fimbria-fornix and medial amygdala are important for regulating investigation of odor and scent marking but not individual discrimination (Petrulis and Johnston 1999; Petrulis et al. 2000). Finally, studies using rodents as models for describing neural circuitry in the mammalian olfactory system (e.g., Belluscio et al. 2002) also complement our understanding of the perceptual mechanisms involved. For example, when mice sniff conspecifics, individual neurons in the accessory olfactory bulb vary in activity depending on the sex and genetic strain of the other mouse, suggesting that populations of neurons may become tuned to recognize specific individuals (Luo et al. 2003). Such approaches hold great promise for the future.

Knowledge about the role of scent marking in modulating social behavior is also being used in designing new approaches to problems in animal welfare and conservation. In the laboratory, the link between scent marks and aggression raises the possibility of adapting husbandry practices to reduce aggressive behavior (Olsson et al. 2003). For example, transferring odor cues during routine cage cleaning may be one way to reduce postcleaning aggression peaks, but research to date is still inconclusive. In one study the transfer of urine-impregnated sawdust reduced aggression (Gray and Hurst 1995), while it intensified in another (Van Loo et al. 2000). Transfer of nesting material, on the other hand, appears to reduce aggression (Van Loo et al. 2000) and concentrations of stress-indicating hormones (Van Loo et al. 2003). In the field of conservation, Sutherland and Gosling (2000) highlighted the potential for increased understanding of processes underlying mate choice, including scent marking, in overcoming behavioral incompatibility and extreme intrapair aggression in captive breeding programs. In harvest mice, Roberts and Gosling (2004) manipulated sexual signaling characteristics of relatively unattractive males to increase their allure to females. Manipulating the degree of familiarity also influenced female preferences and reduced the amount of aggression between mates upon pairing. Once again, rodent studies are a useful model in which to test such ideas with a view to application in other, often more threatened, species such as giant pandas (*Ailuropoda melanoleuca;* Swaisgood et al. 2000).

Summary

Scent marking is an important feature of rodent social behavior. Scent marks are status signals used in assessment by receivers, who are usually same-sexed conspecifics or potential mates but could also be "eavesdroppers" (including predators). Status information carried by scent marks includes resource-holding potential (e.g., territory ownership), social status, health, and hormonal, nutritional, and reproductive condition. Scent markers benefit by reducing contest frequencies, maintaining social status, or attracting mates, and they invest heavily to ensure that their system of marks is maintained and that information carried in them is unambiguous. Receivers are expected to respond adaptively to this information. Because receivers vary in competitive ability or reproductive status, and because the costs and benefits of responding to marks in particular ways vary between individuals at different times, responses to scent marks also vary greatly.

Rodent studies have largely shaped our understanding of scent marks as signals of status. Status signaling is a theoretical paradigm that explains almost all marking behavior across taxa and provides a unifying framework, grounded in evolutionary theory, within which to study scent marking alongside other signals in the visual and acoustic modalities.