Hormonal Correlates of Social Rank in an Asocial Species, the Common Brushtail Possum (*Trichosurus vulpecula*)

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Abstract

Dominance hierarchies play an important role in access to mates or resources in many species. Rank is sometimes correlated with circulating testosterone levels or morphological traits such as body weight. The relationship of glucocorticoid secretion and rank, however, is less clear. In this study, we investigated the relationship of male rank to body weight, circulating testosterone and cortisol concentrations in captive possum triads (*Trichosurus vulpecula*). We carried out two experiments to examine hierarchy formation and the effects of castration of the dominant male during the non-breeding season. A third experiment measured the effects of removal of the dominant male from a stable hierarchy during the breeding season. We found that dominant male rank was significantly correlated with higher circulating testosterone levels during periods of hierarchy formation and during the breeding season but not during periods of hierarchy stability in the non-breeding season. Lack of correlation between plasma testosterone concentration and rank after male castration suggests that stable social rank is not dependent on hormone level and may be more dependent on behavioural traits. Any biocontrol measures that rely on manipulation of hormone levels may be unreliable when applied to unstable hierarchical situations, including the establishment of territories by subadult males, and the pre-breeding season when circulating testosterone concentrations peak in males and a period of hierarchy establishment may occur.

Introduction

Dominance hierarchies may determine access to food, mates and other resources in social living species. Dominance rank classification can be made by recording dyadic relationships (Drews 1993; Forkman & Haskell 2004). In some species, high concentrations of circulating testosterone are good predictors of dominance (e.g. Wingfield et al. 1987, 1990; Knol & Egberink-Alink 1989; Kotrschal et al. 1998; Poisbleau et al. 2005a) particularly during periods of social instability. However, little work has been carried out with asocial species to determine whether similar patterns of endocrine correlates occur. Brushtail possums (*Trichosurus vulpecula*) are nocturnal marsupials generally classed as a solitary species (Bolliger 1944; Winter 1976) but they do have social interactions, especially in the winter breeding season from around March to September (Winter 1976) and around localized food sources. Possums also have overlapping home ranges (Cowan 1990). Clear dominance behaviour is evident and possum hierarchies occur in both small captive groups (Biggins & Overstreet 1978; Oldham 1986; Jolly & Spurr 1996) and in loose aggregates that have been observed in wild possums during the breeding season (Winter 1976; G. Hickling, unpubl. data).

Concern about the impact of introduced brushtail possums on indigenous flora and their role as vectors of bovine tuberculosis has led to the classification of possums as pests of national importance in New Zealand. Among various proposed control strategies is the possibility of modifying the reproductive success of these animals (Barlow 1994, 1997), however, the use of immunocontraception or hormonal contraception to achieve this raises questions about the influence of reproductive hormones on social behaviour patterns (Jolly 1993). The relationship between circulating testosterone concentration and dominance is of particular interest in the possum as biocontrol methods that affect sex steroid levels could have profound effects on behaviour and social status. Modification of reproductive status could in turn have implications for the employment of population control strategies.

This study was carried out to determine whether there is a relationship between social rank and plasma testosterone concentration in male possums; the hypothesis under test being that social rank is positively correlated with plasma testosterone concentration. This hypothesis was addressed in two ways. Firstly, plasma testosterone was monitored in males engaged in formation of a social hierarchy. Secondly, effects on social ranking and plasma testosterone concentrations of dominant and subordinate male possums were monitored following disturbance of the social hierarchy, either by removal of one of the males or by castration of the dominant male.

As the experimental animals were exposed to the combined effects of capture stress and the stresses arising from interactions between individuals, plasma cortisol concentration and, in some cases, packed cell volume of blood were measured as potential correlates of stress in these animals.

Materials and Methods

Animals

Adult possums were trapped from the wild in the Canterbury region of New Zealand and transferred to the Landcare Research field research facility where they were housed in individual cages for approx. 4 wk to habituate them to captivity. All procedures used in this study were approved by the Landcare Research Committee for Animal Welfare. The possums were tagged for identification with metal clips punched through the outer pinna of the ear and were checked daily for signs of ill health or poor body condition. After habituation, possums were transferred to outdoor pens ($4 \times 4 \times 2.14$ m high grassed enclosures) for the experiments, where they were matched by body weight and allocated to 'triads' consisting of two males and one female. They were fed ad libitum a mixed diet of pelleted concentrate feed, fruit and vegetables and had free access to water and shelter.

Bilateral castration (expt 2) was carried out using aseptic procedures while the animals were under halothane anaesthesia. The testes and epididymides were dissected from the spermatic cord via a scrotal incision, then blood vessels tied off and the skin incision closed with braided nylon thread. Comparable control animals underwent a sham castration operation and all surgical subjects received antibiotic by i.m. injection (1 ml Penstrep LA; Bomac Laboratories Ltd, Auckland, New Zealand) post-operatively.

Blood Sampling and Live Weight Recording

Blood sampling and weighing was carried out at the commencement of each experiment and at least once weekly thereafter. Possums were collected during daytime between 16:00 and 18:30 h while they slept in hessian sacks, transferred still in the sack to a large plastic container, and anaesthetized by filling the container with 5% (v/v) halothane vapour (Fluothane, ICI, Auckland, New Zealand) in oxygen. Blood samples (5 ml) were obtained by jugular venipuncture of the anaesthetized animals. A sample was removed for determination of packed cell volume and the remainder transferred to heparinized glass tubes and centrifuged for 10 min (1000 ***g***). Plasma was stored at −20°C. Body weight was recorded when blood sampling occurred.

Hormone Assays

Testosterone concentration of plasma samples was assayed by an enzyme-linked immunosorbent assay (ELISA). This utilized 50 μl aliquots of plasma extracted with diethyl ether. Assay sensitivity was 0.1 nmol/l and intra- and inter-assay coefficients of variation (CV) were 8.7% and <12.5% respectively. Plasma cortisol concentration was measured by the ELISA method of Lewis et al. (1992). This assay had a sensitivity of 5 nmol/l and intra- and inter-assay CV of 5.0% and 9.4% respectively. Both assays were validated for possum plasma prior to measurement of the experimental samples and all samples from an animal were assayed on a single ELISA plate.

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Determination of Rank

The onset of possum activity begins as light intensity declines following sunset (Herbert & Lewis 1999). Dominance relationships were established by observing the animals from 30 min after sunset for usually 2.5 h on three or four evenings a week. On any given night, three to six pens were randomly selected and each observed for 20–60 min from an area outside the pen. Observers made no attempt at concealment but movement was minimized: all possums had been exposed to and at least partially habituated to movement and handling while in the animal house. Dominance status was determined by the paired contest method (Tomback et al. 1989). The outcome of all aggressive behavioural interactions (win or lose) was recorded to determine the dominance status of individuals and classified as threat, chase, fight and/or avoidance. Preliminary testing of inter-observer reliability between the two observers showed 100% concordance, as did further testing 3 mo later.

Experimental Protocols

Studies were conducted to monitor animals during formation of social hierarchies in the non-breeding season (expt 1) and during disturbance of the social hierarchies, either by castration of dominant males prior to the breeding season (expt 2) or by removal of one male animal during the breeding season (expt 3). The breeding season for possums at this facility in Canterbury, New Zealand runs from around April to September/October each year (Jolly et al. 1995).

Experiment 1

Thirty adult possums were matched by body weight and allocated to 10 'triads' consisting of two males and one female held in an outdoor pen ($4 \times 4 \times 2.14$ m high) during November, which is the non-breeding season for these animals. Hierarchy development was monitored by observing behaviour on three or four evenings per week for 6 wk. Observations began half an hour after dusk, and continued for approx. 2.5 h. On any given night three to six randomly selected pens were each observed for 20–60 min.

Experiment 2

During the non-breeding season, in another 13 triads that had been monitored for 8 wk using the methods as above, six dominant males from six pens were castrated and seven dominant males from seven pens were sham castrated. Monitoring continued for a further 6 wk into the breeding season.

Experiment 3

Fourteen further triads were established in the breeding season, monitored for a minimum of 12 wk and the dominance status of individual males was determined as above. Four dominant, four indeterminate and six subordinate males, one from each pen, were chosen randomly for study and the other male in each pen was removed 4 wk later. The study animals were monitored for a further 4 wk to measure body weight and take blood samples only, as there was no possibility of male-to-male social interaction.

Statistical Analyses

Statistical analyses were undertaken using Minitab. Data that were not normally distributed were transformed to their logarithms prior to analysis, which was performed using Student's paired t-test or analysis of variance (ANOVA). Behavioural data recorded during the period of hierarchy development were pooled for individuals during each time period to form one sample point and reduce bias because of repeated measures (Martin & Bateson 1986). Individual data were then grouped according to later dominance status into either 'subordinate' or 'dominant' categories. Time series data were tested using ANOVA. The mean difference between relative testosterone values of dominant and subordinate male pairs (i.e. pair-wise comparisons, Figs 1 and 2) were used in the analysis as there was wide variation in absolute plasma testosterone concentrations between

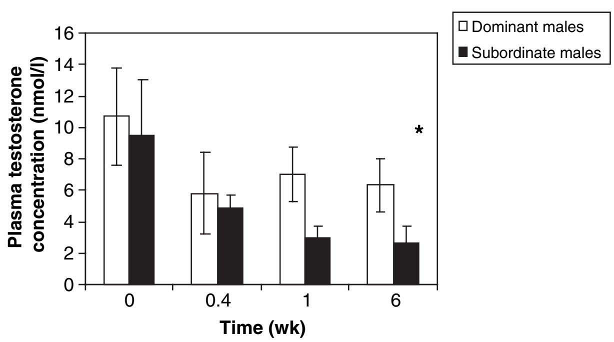

Fig. 1: Plasma testosterone concentration [$\bar{x} \pm$ SE, n = 10 (nmol/l)] of male possums in 10 captive triads during hierarchy formation, grouped according to their subsequently assigned dominant or subordinate status. Significance is indicated by an asterisk ($t_9 = 2.9$, $p = 0.025$)

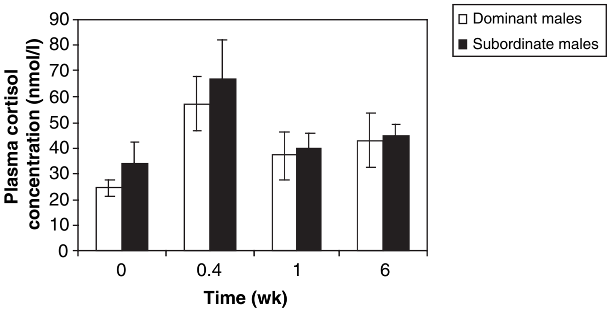

Fig. 2: Plasma cortisol concentration [$\bar{x} \pm$ SE, n = 10 (nmol/l)] of male possums in 10 captive triads during hierarchy formation, grouped according to their subsequently assigned dominant or subordinate status

individuals. The relationship between variables such as testosterone and cortisol concentrations was determined by linear regression analysis.

In the castration and removal experiments (expts 2 and 3), one sample paired Student's t-tests were carried out on the mean differences in testosterone and cortisol values for paired dominant and subordinate possums.

Results

Experiment 1 Hierarchy Formation

There was sufficient paired contest activity recorded between the male possums (win–lose interactions, Table 1) to enable the allocation of males to either dominant or subordinate social-status classification for all 10 triads and it was clear that a stable dominance hierarchy was established in each case.

Mean body weights of dominant and subordinate males were similar at the start of the study ($\bar{x} \pm$ SE: 3.06 ± 0.18 and 3.01 ± 0.21 kg respectively). Males of both groups lost weight during the first 3 d in pens; thereafter, body weights recovered and were not affected by dominance ranking.

Table 1: Numbers of interactions between possum pairs observed during the first 6 wk of establishment of 10 captive triads

Interaction type	Male to male	Female to male
Win–lose		
With physical contact	4 (9)	8 (8)
Chase	11 (20)	13 (12)
Avoidance	34 (63)	59 (56)
Appraisal[a]	4 (7)	19 (18)
Other	1 (2)	7 (7)
Total	54 (100)	106 (100)

Values are given as n (%).
[a]Mainly nose to nose behaviour with no clear winner.

There was no difference between dominant and subordinate males in mean plasma testosterone concentration at the outset of the study (Fig. 1) but the mean concentration of testosterone in subordinate males fell progressively with the passage of time (repeated measures ANOVA on log transformed data: $F_{(3,9)} = 3.58$, $p < 0.03$; Fig. 1). Overall, plasma testosterone concentration of subordinate males was lower (paired t-test on difference: $t_9 = 3.6$, $p < 0.01$) than that of the dominant males they were paired with (Fig. 1). Plasma testosterone concentration in dominant males was not well-correlated with rate of involvement in win-lose interactions (linear regression: $r = 0.61$, $n = 129$, $p = 0.2$).

In general, dominant males had mean plasma cortisol concentrations that were lower than those of the subordinate males (Fig. 2), but paired t-tests on log-transformed data in each time period ($n = 10$) showed that these differences were not significant. Both groups of males had elevated mean plasma cortisol concentrations on Day 1 after placement in triads, concurrent with a reduction in mean plasma testosterone concentration, but a one-way t-test ($n = 17$) showed there was no significant difference between the two groups. However, there was no correlation between plasma testosterone and cortisol concentrations throughout the study.

Experiment 2 Castration of Dominant Males in the Non-breeding Season

Castration had no effect on social status as all six castrated males maintained their dominance ranking. One non-castrated male lost dominance during the post-castration period. In this experiment, mean plasma testosterone concentrations were low and were not affected by dominance status of the possums (Fig. 3). Castration almost eliminated circulating concentrations of testosterone (Fig. 3). In both groups of intact possums, testosterone levels increased significantly during the course of the study (repeated measures ANOVA for control and treated groups pooled: $F_{(3,240)} = 7.69$, $p < 0.001$). In non-castrated possums, there was no relationship between the plasma concentrations of cortisol and testosterone (linear regression on log transformed data, $r^2 = 0.0$, $p = 0.4$). Cortisol did not vary significantly during the course of the study (repeated measures ANOVA: $F_{(3,252)} = 3.97$, $p = 0.09$). Castration had no effect on plasma cortisol concentration of castrated males relative to intact males (two-way

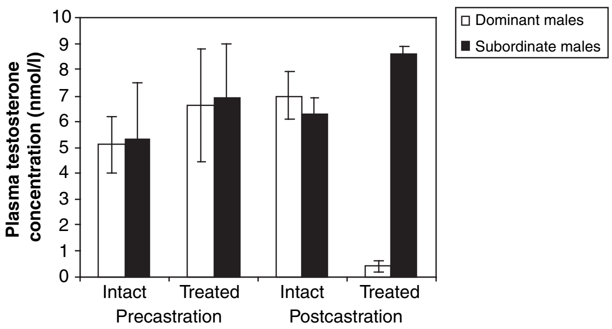

Fig. 3: Plasma testosterone concentration [$\bar{x} \pm$ SE (nmol/l)] of dominant male possums and their paired subordinates pre- and post-castration of six of the dominant males

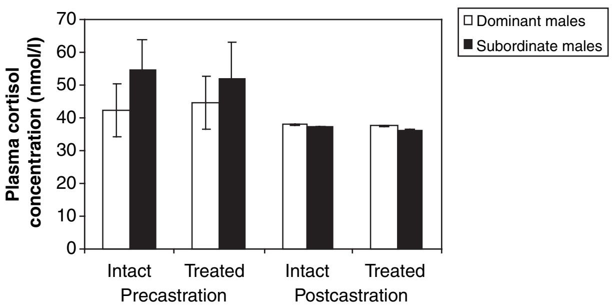

Fig. 4: Plasma cortisol concentration [$\bar{x} \pm$ SE (nmol/l)] of dominant male possums and their paired subordinates pre- and post-castration of six of the dominant males

ANOVA, $F_{(1,342)} = 1.05$, $p = 0.31$, Fig. 4). Likewise, there was no change in packed cell volume of blood samples (range 30–50% with most values between 35% and 45%).

Experiment 3 Removal of Dominant Males in the Breeding Season

Dominant males had a consistently higher mean plasma testosterone concentration than subordinate males, both before and after the removal event (ANOVA on log-transformed data, $F_{(1,77)} = 7.2$, $p < 0.01$). Mean plasma testosterone concentration was not affected by removal of the experimental males. There was no effect of social status on mean body weight, and body weight was not affected by removal of one of the males from each triad.

Discussion

Dominance Rank and Testosterone

The results show, for the first time, a significant direct relationship between circulating testosterone concentration and rank in male possums during periods of hierarchy formation, and in the breeding season. At the time of placement in triads, there was no difference in plasma testosterone concentration between males who became dominant and those who later became subordinate. However, a difference became pronounced, and statistically significant, as hierarchy formation continued. This is consistent with other studies showing that rank-related testosterone concentrations may occur, or are more pronounced, in unstable situations (e.g. Wingfield et al. 1987, 1990; Sapolsky 1993; Brockman et al. 1998; Klinkova et al. 2004).

Although the evidence for an overall relationship between interaction rates and plasma testosterone concentration was weak (they were poorly correlated), there was a period during the formation of the social hierarchies (second and third week) when dominant males were involved in 80% and 77% of win–lose interactions respectively. The relationship between win–lose interactions and testosterone levels was less clear, and although the existence of a link between social behaviour and circulating testosterone levels in male possums has been suggested by other studies (Oldham 1986) the findings reported here do not reinforce this view. There was also apparently no effect on intensity and frequency of possum interactions. Olfactory cues such as marking may also be important to reinforce social rank in nocturnal animals such as possums, and it is possible the lack of frequency or intensity in possum interactions may reflect this.

In this study, the relationship between social ranking and plasma testosterone concentration was not universal. In the initial experiment, during a period of hierarchy instability, plasma testosterone concentration fell progressively with time but it was generally higher in the males who became dominant. Likewise, dominant males had higher plasma testosterone concentrations than both subordinates and animals with an indeterminate rank in the breeding season when the concentrations were relatively high. This difference between dominant and subordinate males was not apparent in expt 2 in the non-breeding season. This suggests that testosterone and dominance are more important during the breeding season, perhaps to secure access to mates. Certainly, wild possums are more likely to interact in the breeding season, and endocrine status which is related to male dominance may therefore confer an advantage on certain males in their access to females.

Subsequent disturbance of stable social groups by removal did not alter plasma testosterone concentration and the lowering of testosterone concentration by castration also did not affect social ranking. These latter findings indicate that once social status has been established, the relationship with circulating testosterone concentration may cease to apply. Behavioural traits therefore appear to maintain male dominance rank.

Although some studies have indicated there may be correlations between body weight and circulating hormone levels (Allen & Aspey 1985; Lott & Galland 1987), others have not (Poisbleau et al. 2005a). There was no correlation between testosterone and body weight in this study, and body weight was not a predictor of dominance. The results rule out body weight and stress (determined from plasma cortisol concentration and packed cell volume of blood) as important factors in the relationship between social ranking and plasma testosterone concentration in male possums.

Plasma testosterone levels were similar in both dominant and subordinate males in the stable hierarchies during the non-breeding season (expt 2) in contrast to the findings in the hierarchy formation period. This suggests that during periods of instability there may be a different physiological response by dominant and subordinate males leading to different plasma testosterone concentrations. One interpretation of this is that, although the ability of one animal to dominate another may be predetermined, it is when animals have opportunity to experience their social position that an endocrine response manifests as stimulation or suppression of the reproductive axis on some occasions. According to these findings plasma testosterone concentrations do not appear important in maintaining dominance during stable periods in the non-breeding season. The lack of difference in plasma testosterone levels of dominant and subordinate males during the non-breeding season also supports the hypothesis that high plasma testosterone concentrations are not a necessary correlate of dominance during this period.

The higher mean values for plasma testosterone concentration in the breeding season compared with the non-breeding season (Fig. 4) are consistent with seasonal changes previously reported in brushtail possums (Gemmell et al. 1986). However, during expt 3 in the middle of the breeding season, dominant males had significantly higher testosterone concentrations than subordinate males, and indeterminately ranked males had very low plasma testosterone concentrations. The difference in testosterone for dominant and subordinate males during the breeding season is consistent with a number of other studies (e.g. Guillemain et al. 2000; Poisbleau et al. 2005a) and it has been suggested that this may be related to potential challenges by conspecific males for mates (e.g. Johnsen 1998; Cavigelli & Pereira 2000; Muller & Wrangham 2004). In possums, the mating system seems to be highly variable. In one captive study from New Zealand only dominant males successfully bred (Jolly et al. 1999), but other studies have shown variable degrees of polygyny (Taylor et al. 2000; Clinchy et al. 2004), which may be related to both seasonality and density (Isaac & Johnson 2003).

There is some suggestion in the literature that during the breeding season plasma testosterone concentrations are suppressed in subordinate males, including possums (Gemmell et al. 1986; Kerle & Howe 1992). In both of these cases, the authors speculated that suppression was because of the presence of a dominant male. If this were the case, removal of dominant males from triads should have resulted in increased circulating testosterone concentrations in remaining subordinate males. This would have implications for the efficacy of possum control programmes that tended to remove dominant males, as induced breeding by subordinates would tend to counteract the benefits of such control programmes. However, the results obtained here show no evidence of any response in plasma testosterone concentrations of the subordinate males nor any effect on hierarchy. Removal of dominant males from triads had no effect on circulating testosterone concentrations in subordinate males. Van de Crommenacker et al. (2004) found that subordinate male Seychelles warblers had low testosterone during the sexually active period, and suggested that they do not invest in elevated testosterone levels as they rarely mate successfully. This appears to be a useful hypothesis for investigation in male possums. In triads where the subordinate male was removed one might expect that plasma testosterone concentrations of remaining dominant males might not remain high in the absence of other males, if this rise was associated with male–male aggression or the presence of 'challengers'. However, no significant difference was detected in the present study. These data do not support the 'challenge' hypothesis, which suggests that elevated testosterone in dominant males may be the result of potential for competition for mates (Wingfield et al. 1987).

Cortisol

There was no statistically significant difference in plasma cortisol concentrations of differently ranked possums during the trials, although subordinate males tended to have slightly higher values than dominant males. However, the literature is so far inconclusive on the relationship between glucocorticoids and rank. Creel (2001) reviewed social dominance and stress hormones and noted that much of the early research compared the glucocorticoids of winners and losers and showed that losing fights can increase glucocorticoid secretion, a general response to stress (e.g. Louch & Higginbotham 1967; Manogue 1975). That is, they provided evidence that social subordination is stressful. Other later studies have reached similar conclusions (e.g. Schwabl et al. 1988; Sloman et al. 2001). As well, increased glucocorticoid concentrations have been correlated with a reduction in reproductive behaviour or in hormones associated with reproduction (Wingfield et al. 1994; Blanchard et al. 2001). However, more recent studies have suggested that dominant males may suffer higher stress (e.g. Creel et al. 1997; Johnsen 1998; Pravosudov et al. 2003; Sands & Creel 2004; Mateos 2005) or have found no difference in glucocorticoids between dominants and subordinates (e.g. Wingfield et al. 1991; Creel et al. 1992; Robbins & Czekala 1997; Schoech et al. 1997; Poisbleau et al. 2005b). Sands & Creel (2004) argued that it is not logically necessary for social stress to be greater for subordinates than dominant animals, and noted that many, although not all, of the earlier studies were carried out in captivity. The suggestion is therefore that the relationship between basal glucocorticoid concentrations and rank is highly variable, and sources of variation include the social system and whether the study was conducted in captivity. The lack of correlation between plasma cortisol and testosterone concentrations indicates that control of the reproductive axis in males may not be strongly linked to control of the stress axis.

Behaviour

Interaction rates in the breeding season were higher than in the non-breeding season, consistent with Winter's (1976) study of wild possums. Although higher interaction rates have been recorded elsewhere during the non-breeding season (Day et al. 2000), as the authors themselves suggest, these data are most likely an artefact of how interactions were quantified.

Prior to castration (expt 2), there were no differences between the control and treated groups in interaction rates, marking rates, interaction intensity or the proportion of male–male or female–male interactions. However, during the transition between the hierarchy formation period and this stable period, interaction rates were higher although the proportion of win–lose interactions was similar. There were very few differences between the treated and control groups after castration. Hierarchy stability was unaffected by the castration event, and both the interaction rates and marking rates remained similar to the pre-castration values. Pinxten et al. (2000) castrated male European starlings and suggested that maintenance of aggressive behaviour after castration of male adults may result from experience or action of non-gonadal sex steroids. Certainly, the results obtained here support behavioural maintenance of a stable hierarchy.

Our results suggest that although possums are solitary marsupials, circulating testosterone is important in the determination of male rank during periods of hierarchy instability. This might include the establishment of new territories in the wild. The significant relationship between testosterone and male rank during the breeding season suggests that rank may be important for access to females. Further studies of the possum mating system are needed to clarify this relationship.

Implications for Biological Control

Although the results of expt 2 suggest that castration or reduction of testosterone in male possums may be a suitable technique for possum biological control, this study used adult sexually mature males in stable hierarchies in the non-breeding season. However, if there are critical periods in the life cycle where testosterone influences possum behaviour, such as the migration of young immature males into new territories, or the formation of male hierarchies prior to breeding, there may be unforeseen effects resulting from these biological control methods. Thus, it would be unwise to assume that the present results can be applied to biological control programmes in the wild. In addition if testosterone production is suppressed, this may in turn affect male muscle mass and body weight and thus alter the ability of males to defend resources and status.

If dominance hierarchies play a role in natural mating, there are important implications for the spread of biological control agents (Barlow 1997). Biological control methods in the possum that affect hormonal secretion in a similar manner to castration would rely on behaviours and hierarchies being relatively unaffected by these procedures, as this is a pre-requisite for their success.

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