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Weak winner effect in a less aggressive mammal: Correlations with corticosterone but not testosterone

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Abstract

We investigated the existence of the “winner effect” (winning an aggressive encounter following previous victories) and an associated rise in testosterone (T) in the white-footed mouse (*Peromyscus leucopus*) which generally display low levels of aggression and territoriality. We compared the effect of previously winning three, two, one, or zero resident–intruder encounters on the likelihood of winning a subsequent aggressive encounter. Although 50% of males were removed during training because of peaceful encounters, the winner effect was weak and not significant. We hypothesize that territoriality/aggression may be associated with the strength of the winner effect and discuss whether the slight winner effect exhibited by *P. leucopus* may become significant when population densities increase and males become more territorial. There was also no associated change in T with winning; however, corticosterone (Cort) changed with experience as winners had low Cort levels compared to losers and controls. Furthermore, low Cort levels in winners were associated with quicker attack latencies. These results contrast with findings of a significant winner effect and increase in T in males of the highly territorial and aggressive California mouse (*Peromyscus californicus*) using an identical methodology. California mice also attacked their opponents at more caudal regions of the body compared to white-footed mice that attacked their opponents at more rostral regions of the body, possibly related to different levels/types of aggression expressed by the two species.

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Keywords: Winner effect; Testosterone; Corticosterone; *Peromyscus leucopus*; Rodent; Mammal; Offensive aggression; Defensive aggression; Challenge effect

1. Introduction

A winner effect, the increased probability of winning an aggressive encounter following previous victories, has been demonstrated in a wide variety of species including both non-mammalian and mammalian such as fish [1,2], reptiles [3], insects [4–6], and mice [7], although a number of studies have found no evidence of a winner effect at all [8–10]. A review of winner effect studies [2] provides some explanations for the variance in these findings. One obvious source is variation between species because of mating systems and other associated variables. Some inconsistencies may stem from methodological differences such as random selection versus self-selection (the winner in an encounter is assigned to the winner group and the loser is assigned to the loser group), which could confound results due to genetic

predispositions of self-selection [2]. The existence of the winner effect may also be difficult to capture because of the nature of its expression. Several studies have shown that the winner effect is fairly short-lived, disappearing over a period of time ranging from seconds to several days depending on the species [2,7,11–13]. As a consequence, a winner effect may be found at one time point, but not at another. It is conceivable that other species differences are related to the costs and benefits of fighting, including the risk of injury and the probability of aggressive social interactions occurring [1,3,7]. Thus, an appreciation of the natural history of the study species may be helpful in understanding the occurrence of the winner effect. Hypothetically, a species with an ecological system that is very territorial and very aggressive may be more likely to show clear evidence of the winner effect. To establish and maintain a territory a species may need to learn and remember past winning and losing experiences (location, opponent characteristics, fighting strategies, etc.). However, the winner effect may be less evident in a species that is less territorial and less aggressive, since loss of a territory is not likely to ensue [14]. As a

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consequence, the evolutionary development of this winning skill may be absent or diminished [15].

We previously found a winner effect in male California mice (*Peromyscus californicus*) [7], which have a social system that is very aggressive, territorial, monogamous, and paternal all year round [16]. In the present study we investigated the winner effect in a mammal with a relatively non-aggressive social system, the closely related white-footed mouse (*Peromyscus leucopus*). The polygynous and non-paternal white-footed mouse has a social system that is generally less aggressive and less territorial than California mice [17].

A relationship between aggression and numerous hormones has been well documented. Testosterone (T) and corticosterone (Cort), in particular, are often investigated in relation to winning behavior. The Challenge Hypothesis, which shows an increase in T after a single winning experience, has been well documented as a phenomenon that occurs in birds [18,19], primates [20], and humans [21]. The Winner-Challenge Hypothesis [7] suggests that the increase in T may be causally associated with the ability to win future aggressive encounters (the winner effect). If this is so, one prediction might be that increases in T are less likely to occur in a species with a weak or non-existent winner effect phenomenon, as may be expected in species such as *P. leucopus*. In addition, the males of the *P. leucopus* species have higher baseline T levels that may be closer to their physiological maximum [22,23] and furthermore are polygynous which is predicted to be associated with lower androgen responsiveness [24–27]. As a consequence, changes in T levels may be small and insufficient to achieve a perceptible winner effect.

In contrast to T, the relationship between Cort levels and winning/aggression is very inconsistent. Negative associations with aggression have been found in rodents [27–30] and dominant/subordinant hierarchies in primates [31] and rodents [32], but positive associations with aggression have also been found in reptiles [33], birds [34] and dominant/subordinate hierarchies in primates [35,36]. Others find no association in primates [37], reptiles [38], and birds [39]. We hypothesize that the expression of a weak winner effect, as may occur in a less aggressive and less territorial species, may be associated with a change in the type of hormones, i.e. decreased role of T, that significantly influence aggression. As a result, the role of other hormones such as Cort, may be more apparent when examining changes in aggression.

In this study, our overall aims were to determine whether the winner effect occurs in a species with low levels of aggression and territoriality and, if so, whether these are associated with changes in Cort or T levels linked with winning and aggressive behavior. In addition, we briefly compare two related species, *P. leucopus* and *P. californicus* species in terms of the winner effect, winning behavior, and associated hormones.

2. Methods

2.1. Subjects

Sexually naïve male *P. leucopus* mice were reared in a laboratory colony at the University of Wisconsin, Madison.

Mice were maintained in accordance with the recommendations of the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*. Colony rooms were kept under a 14 h light/10 h dark light cycle with lights on at 0300 CST. After weaning, mice were housed in same-sex groups of three or four mice in standard cages (30×19×13 cm³) and fed Purina 5001 mouse chow and water *ad libitum*. All of the mice in the experiment ranged in age from 6 to 12.5 months. No siblings were used in the same treatment conditions of this experiment. One week prior to the beginning of this study, mice were maintained under a 14 h light/10 h dark reverse light cycle with lights on at 2300 CST. Experimental observations were conducted under dim red light during the early part of the dark phase, between 1330 CST and 1630 CST. A total of 181 male mice were used, of which 50 were test subjects, another 50 were final intruders during the testing phase, 32 were intruders during the training phase, and 49 males were excluded because they peacefully interacted with the intruder during the encounter and therefore did not meet the criteria for a winning experience (see below).

2.2. Behavioral testing

2.2.1. General procedures

To facilitate comparisons the procedures used were identical to those in our *P. californicus* study [7]. We used the same sample size of 10 per group. Eighty-nine *P. leucopus* males were originally randomly assigned to win either zero, one, two, or three times during the training phase, however, 0, 11, 17, and 21 males, respectively, were removed from each treatment group. The experiment was completed when forty males total were retained in the study ($N=10/\text{condition}$, see Table 1). An additional ten males were randomly assigned to a group that experienced no training or test encounters and served as a hormonal control (see Hormonal measurements). In the training phase (see below), each of the forty males was exposed to either an aggressive encounter or a control (handling) experience for a total of three training experiences (Table 1). Thus all individuals were exposed to every disturbance associated with an aggressive encounter other than the presence of the intruder [40].

On Day 1, subjects were weighed (mean±S.E.: 28.9±1.03 g) and paired with a female. On Day 11, each sexually experienced male and its mate were placed in a large glass observation cage. All aggressive trials (Days 13, 15, 17, and 19)

Table 1
Fifty males were randomly assigned to five conditions

Treatment group	Training phase			Testing phase
	Day 13	Day 15	Day 17	Day 19
Control	Ø	Ø	Ø	Ø
Condition 1	Ø	Ø	Ø	Test
Condition 2	Ø	Ø	Win	Test
Condition 3	Ø	Win	Win	Test
Condition 4	Win	Win	Win	Test

Conditions 1–4 were analyzed for behavioral and hormonal measures. Control condition was necessary for hormonal measures only.

followed the resident–intruder paradigm [41], in which the resident and mate were housed at the encounter site 48 h prior to the first experience (Day 11). The female was removed 15 min before each trial. The intruder was introduced to one side of the cage and the removal of a divider signaled the beginning of the encounter. We videotaped the resident and intruder interacting for 10 min. For each individual, the frequencies of biting, chasing, wrestling bouts, jumping away, and retreating, as well as the total time spent freezing were recorded. Attack latency of the winner and freeze latency of the loser were also recorded. The winner/loser status was established when at least three attacks (biting, chasing, or wrestling) occurred during the trial, consistently initiated by the same individual and eliciting avoidance behavior (jumping away, retreating, or freezing) from the opponent. In the training phase, only trials in which a clear winner/loser relationship was established were included in the study. Clear winner/loser status was not established in 49 of 89 training sessions and these trials were excluded from the study. No encounter resulted in obvious injury to the participants.

2.2.2. Training phase

To increase the probability that residents had only winning experiences during the training phase, they were selected to be larger than the intruder (mean difference $\pm$ S.E.: 3.3 ± 0.8 g), sexually experienced, and fully alert. Their unfamiliar opponents during the training phase had the disadvantage of being smaller, sexually inexperienced, mildly sedated using i.p. injections of methotrimeprazine – 5 mg/kg [42,43], and former losers of 1–2 aggressive encounters prior to the experiment. Each intruder was randomly assigned to its resident opponent and was used no more than twice, each time with a different opponent.

2.2.3. Testing phase

To test for the winner effect (Day 19), the established winners, as well as those residents who had zero previous wins, were exposed to an aggressive encounter in which the prior treatment of the intruders was more similar to that of the winners. These unfamiliar intruders were slightly larger than the residents (mean intruder advantage $\pm$ S.E.: 3.7 ± 0.6 g); sexually experienced; fully alert; and had won a previous aggressive experience 1 day prior to the test encounter. The outcome of this interaction (residents won or lost) was used to evaluate the occurrence and strength of the winner effect. Each of these final intruders was randomly assigned to its resident opponent and was used only once.

2.3. Hormonal measurements

Fifty baseline blood samples were taken one week before the training phase through the retro-orbital sinus within 2 min (mean $\pm$ S.E.: 42.7 ± 2.3 s). A second blood sample (trunk blood) was collected 45 min after the final test encounter by rapid decapitation. The 45 min interval was chosen (a) because the only study using rodents demonstrating a change in T found an increase in post-aggression T level at 45 min in male guinea pigs [44] and (b) T increases at 45 min after an aggressive

encounter in *P. californicus* [45]. Samples were immediately centrifuged; the plasma was obtained and stored at -80°C until assayed. The samples were analyzed via enzyme immunoassay for T (T antibody is R156, University of California–Davis diluted to 1:35,000) and radioimmunoassay for Cort [46,47]. For T levels, the intra- and inter-assay coefficients of variation were 5.3% and 5.4%, respectively ($n=3$ plates). For Cort levels, the intra- and inter-assay coefficients of variation were 2.8 and 5.9%, respectively ($n=2$ assays).

2.4. Statistical analyses

All statistical analyses were performed using SPSS Version 11.5. Overall, the behavioral data from resident winners and resident losers of the final encounter were analyzed together and separately.

The winner effect was analyzed using Fisher's Exact Test. Apart from attack latency and freeze latency, behaviors during the final encounters were analyzed by combining treatment groups such that individuals that had no wins or just one win before testing were placed in a low experience group, while individuals that won twice and three times before testing were placed in a high experience group. These treatment groups were combined because when treated separately the frequency differences of the behaviors in the separate treatment groups were less likely to reach significance [48]. One-way ANOVA, one-way ANCOVA, *t*-tests, and Fisher's LSD post-hoc tests were used to analyze all behavioral and hormonal data. We also used Pearson's rank correlations to investigate associations between the behaviors of the test encounter and post-encounter hormonal levels.

3. Results

3.1. Behavioral data

3.1.1. The winner effect (analysis of resident winners and resident losers together)

In these *P. leucopus* males, multiple winning experiences did not significantly increase the probability of winning a future encounter in *P. leucopus* males ($\chi^2=6.72$, $df=3$, $P=0.67$, Fig. 1). However, we did find a pattern for increasing number of wins with increased experience and it is possible that either larger sample sizes and/or the addition of groups with more experience could have resulted in a significant winner effect. Nonetheless despite using the same methodology as with the California mice [7], no significant winner effect was detected.

Resident attack latency in this species was, however, significantly decreased with multiple winning experiences ($F(3,36)=8.33$, $P=0.002$, Fig. 2). Fisher's LSD post-hoc test revealed that males with three previous winning experiences had significantly shorter attack latencies during the final encounter than males with no ($P=0.001$), one ($P<0.049$), or two ($P<0.043$) previous winning experiences. There appeared to be a cumulative effect of prior winning experience on attack latency, however an analysis of attack latency of males with zero, one and two prior winning experiences did not reach

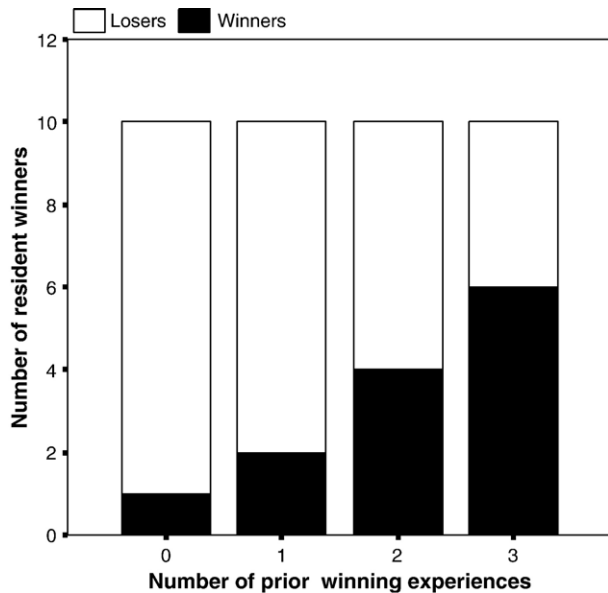


Fig. 1. The winner effect. The percentage of males that won the final encounter across the four conditions ($N=10$ per condition).

significance ($F(2,27)=1.85$, $P=0.177$). The number of prior winning experiences did not significantly alter any other behaviors observed during the aggressive interactions (Biting: $F(3,36)=1.78$, $P=0.17$; Chasing: $F(3,36)=1.89$, $P=0.15$; Wrestling: $F(3,36)=0.30$, $P=0.82$; Freezing: $F(3,36)=1.08$, $P=0.370$; Jumping away: $F(3,36)=2.02$, $P=0.128$; Retreating: $F(3,36)=2.30$, $P=0.093$). However, when winners are combined into a low group (0 and 1 prior winning experiences) and high group (2 and 3 prior winning experiences), individuals in the high group bite more ($T(1,38)=2.24$, $P=0.031$) and chase more ($T(1,38)=1.92$, $P=0.045$) than individuals in the low group. In addition, when encountering residents in the high group, intruders froze sooner (freeze latency) compared to those with low group opponents ($T(1,38)=2.206$, $P=0.034$).

Predictably, variation in the behaviors studied was significantly related to encounter outcome: winners (residents) bit,

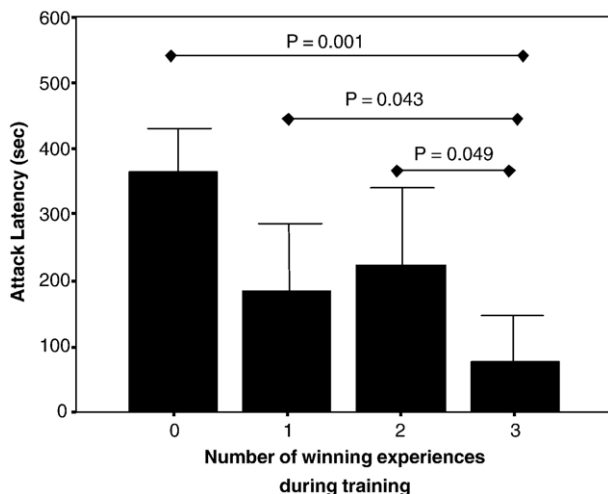


Fig. 2. Attack latency during the final encounter across the four conditions of prior winning experience. Data presented as means $\pm$ standard errors.

chased and initiated wrestling more than losers (intruders) and losers froze, jumped away, and retreated more than winners (Biting: $T(1,38)=3.44$, $P<0.001$; Chasing: $T(1,38)=3.41$, $P=0.002$; Wrestling: $T(1,38)=3.27$, $P=0.002$; Freezing: $T(1,38)=3.12$, $P=0.003$; Jumping away: $T(1,38)=3.92$, $P<0.001$; Retreating: $T(1,38)=2.05$, $P=0.031$).

3.1.2. Resident winners and resident losers

To this point, we have grouped data on residents together, irrespective of whether they were winners and losers. When we analyzed the behavioral data separately by resident winners and resident losers, we found no significant results across the 4 conditions.

3.2. Hormonal data

3.2.1. Testosterone levels

We found no differences between T levels in winners, losers, or controls ($F(2,46)=0.52$, $P=0.600$, see Fig. 3). In addition, an ANCOVA revealed no relationship between T levels and variation in the amount of prior winning experience ($F(4,46)=1.06$; $P=0.387$).

3.2.2. Corticosterone levels

There was significant variation in Cort levels in different treatment groups. The model that best explained the variation in Cort levels (adjusted $r^2=0.292$; $F(7,39)=2.19$, $P<0.041$) incorporated resident age ($F(1,39)=2.08$, $P=0.224$) as a covariate. Analyses using a one-way ANCOVA ($F(2,44)=3.23$, $P=0.029$) revealed that Cort levels in resident winners were significantly lower than the levels of resident losers and controls ($P=0.015$ and $P=0.036$, respectively; see Fig. 3).

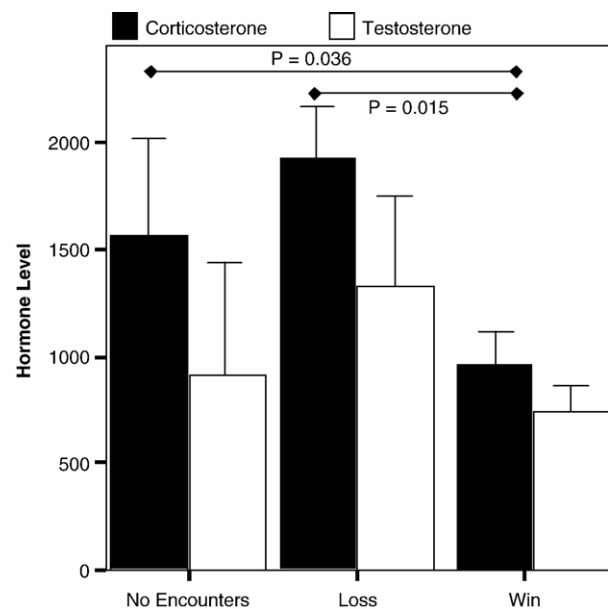


Fig. 3. Corticosterone (ng/ml) and testosterone (pg/ml) levels in controls, all resident losers, and all resident winners. Corticosterone levels are decreased in resident winners compared to controls and resident losers. Data presented as means $\pm$ standard errors.

A reanalysis of data in the related *P. californicus* species [7], using methods identical to those in the current study, also identified a non-significant trend, using resident age and time to obtain blood sample as covariates, suggesting that Cort levels in animals displaying the winner effect are lower than those of resident losers (overall ANOVA: $F(2,48)=1.45$, $P=0.204$; LSD comparing losers to winners: $P=0.087$).

3.2.3. Interactions between hormones and behavior

Regarding T, no behaviors recorded prior to the final blood sample were significantly correlated with either baseline or post-final encounter T levels. Regarding Cort, only in resident winners was there a positive partial correlation between post-final encounter Cort levels and attack latency ($r=0.566$, $n=13$, $P=0.045$, Fig. 4), after controlling for baseline Cort levels and age. In contrast, no significant relationship was found between Cort levels and attack latency in resident losers examined both with and without the outlying data point (with data point: $r=0.232$, $n=27$, $P=0.365$; without data point: $r=-0.322$, $n=26$, $P=0.162$; Fig. 5).

3.3. Interspecies comparison

A high percent of *P. leucopus* resident males (55.1%) investigated interacted peacefully or avoided each other and were removed from the study because they did not win and displayed non-aggressive behaviors during the aggressive encounter without obviously winning or losing. We compared this finding to the percent removed (0.07%) in an identical study in a related species, *P. californicus* [7] and found that a significantly higher percentage interacted peacefully in the present study ($\chi^2=75.8$, $df=1$, $P<0.001$). When we compared topography of attacks in the two species we found that *P. leucopus* resident males attacked more on the snout and face (51.5% of total attacks; the target of attack was not influenced by winning experience, $r=0.21$, $P=0.45$). Furthermore there was no change in the balance of attack targets with different levels of experience ($r=0.33$, $P=0.37$). In contrast, a reanalysis of the videos taken in the *P. californicus* study [7] found that resident

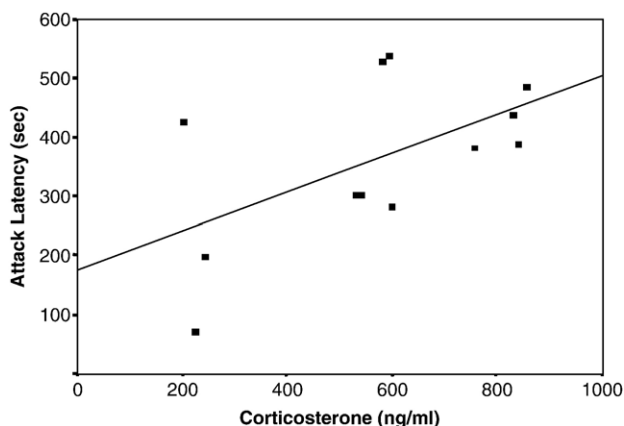


Fig. 4. Relationship between corticosterone levels and attack latency during the final encounter in resident winners only.

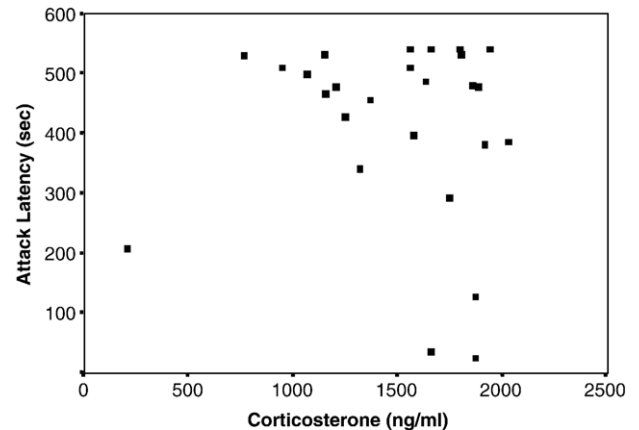


Fig. 5. Relationship between corticosterone levels and attack latency during the final encounter in resident losers only.

males attacked more on the back and flanks (77.33% of total attacks).

4. Discussion

In contrast with the territorial *P. californicus* [7], we found no significant winner effect in male *P. leucopus* mice, perhaps as a correlate of the species differences in territoriality and aggression. We hypothesize that transient changes in T might also contribute to these varied findings because *P. californicus* males had a significant rise in T in association with winning [45,73], while we found no such change in *P. leucopus* males, at least at one time point. The hormonal findings of *P. leucopus* also revealed a significant decrease in Cort levels compared to both losers and controls. This finding is akin to that of *P. californicus* in which a non-significant trend revealed a similar pattern.

4.1. Behavior

The winner effect was slight and not significant in *P. leucopus*. As a species with low levels of aggression and territoriality, the *P. leucopus* male mice had only a 60% chance of winning the final encounter after three previous winning encounters (0 wins: 10%, 1 win: 20%, 2 wins: 40%, 3 wins: 60%), compared to 100% in *P. californicus* (7) (0 wins: 30%, 1 win: 50%, 2 wins: 70%, 3 wins: 100%). Because of the significantly larger percentage of individuals removed from the study (55.1% compared to the 0.07% removed from the *P. californicus* studies) due to low levels of aggression a self-selection process ensued, such that the more aggressive of the *P. leucopus* males remained in the study. In spite of this confound, the winner effect was still not statistically significant. In *P. californicus* on the other hand there was a strong winner effect that lasted for more than 2 days, despite employing identical methodology and sample sizes [7]. The pattern of winning in *P. leucopus* males does suggest, however, that there may be a cumulative effect and a significant winner effect might emerge if the sample size or number of previous winning experiences prior to the final encounter was increased. Such a result might

also lead to the speculation that the winner effect would be expressed in white-footed mice when they perceive population densities as being high, at which point *P. leucopus* males do become territorial under field conditions [74] when they may switch from using a mobile search strategy to find females to a more localized strategy [75]. Regardless, it is clear that the winner effect is weaker compared to *P. californicus* [7], a species that is more territorial and aggressive.

Although there was no apparent winner effect in *P. leucopus*, aggressive behavior did significantly change with prior winning experience. Attack latency decreased with more prior wins. Also biting and chasing behavior increased more after two and three prior wins combined than after zero and one win combined. In addition, resident males with a high level of winning experience elicited losing behavior (freezing) from the intruders sooner than residents with a low level of winning experience. But despite these significant indications of increased aggression, no significant winner effect emerged, suggesting that aggressive or dominance behavior may be varying along more than one dimension.

4.2. Hormones

Testosterone did not change in *P. leucopus* at 45 min after experiencing the final encounter as it did in *P. californicus* [45]. Thus the Challenge Effect did not occur at that time point. Although support for the Challenge Hypothesis has been well documented (e.g. [19,20,49]) there are several other studies that have not found a significant rise in T in association with aggression (e.g. [50–52]). Androgen responsiveness is predicted to be low in polygynous species, such as *P. leucopus*, and high in monogamous species, such as *P. californicus*, as has been demonstrated using a phylogenetic analysis in birds extending predictions of the Challenge Hypothesis originally proposed by Wingfield and colleagues [24–27] and is supported by studies with cichlid fish [27]. Furthermore, our study with *P. leucopus* is also consistent with bird studies finding that species with high baseline levels of T are less likely to have a significant change in T in association with aggression [53,54]. In a small comparison of *Peromyscus* species we found *P. leucopus*, *Peromyscus maniculatus* and *Peromyscus polionotus* to have higher levels of baseline T than *P. californicus*, *Peromyscus aztecus*, and *Peromyscus eremicus* [22], suggesting that *P. leucopus* maintains a higher level of baseline T. One inconsistency between our *Peromyscus* studies and the bird studies is that *P. leucopus* does not express high levels of aggression and the bird studies would tend to indicate that maintaining high levels of T is more consistent with high levels of aggression. This may be complicated, however, in that our samples were obtained from mice under laboratory conditions and thus represent actual breeding baseline levels as opposed to levels that might occur when males are frequently challenged.

In *P. leucopus*, it is possible that these explanations may be the primary reasons to justify the lack of change in T in association with aggression. However, it is also possible that the Challenge Hypothesis was not corroborated because the plasma samples were collected at a less ideal time point if 45 min does

not coincide with the peak rise in T (i.e. see Ramenofsky [72] and Summers et al. [71] for more rapid effects in birds and lizards). This time point was selected because it is the time point at which T peaked after a winning experience in a related species, *P. californicus* [7]. It is thus likely that this time point was appropriate, however, it was not directly tested.

Corticosterone, on the other hand, was significantly associated with winning behavior in *P. leucopus* males. Resident winners of the final encounter had significantly lower Cort levels compared to resident losers and controls (residents who experienced no encounters). There is a hint of a similar pattern in *P. californicus* by way of a non-significant trend for lower Cort levels in resident winners. Some studies have reported higher Cort levels in association with aggression and winning [55,56], and others lower [27–29,32]. Furthermore, in a study of rats, residents that showed high levels of aggression in response to an intruder entering their territory failed to mount an ACTH response compared to residents that were not aggressive to an intruder [30]. Our results are somewhat similar to these. The inconsistencies in the direction of change in Cort levels are yet to be clarified and may be species-specific. In some rodent species, male plasma Cort levels rise with affiliative behavior [57]. It is thus possible that non-affiliative behavior can lead to lower Cort levels (as others have suggested [31]). It is also possible that low Cort levels in association with winning are secondary to the two extremes of a general stress response to aggression. One an active behavioral response, which is fight or flight; and the other a passive response that is conservation withdrawal [58]. Active behavioral stress response patterns are associated with high sympathetic activity, but low Cort levels; while passive behavioral stress responses are associated with high parasympathetic activity and high Cort levels [59,60]. Thus, individuals who win may have an active behavioral stress response pattern with high sympathetic activity and low Cort levels [61,62]. Thirdly, it is also possible that the *Peromyscus* social system is more stable and predictable, so that dominant individuals that won consistently have lower Cort levels compared to individuals that lost [31]. And finally, as already stated, there might have been an earlier transient rise in Cort with a return to baseline by the 45 min time point of plasma sampling.

4.3. Hormonal and behavioral correlations

Although changes in T levels were not associated with any behavioral changes, Cort levels were correlated with attack latency in resident winners of the final encounter, such that residents that attacked their opponents faster had lower Cort levels. On the other hand, this hormonal and behavioral association present in resident winners was not found among resident losers. Recent research investigating attack latency in relation to Cort levels found individuals selected for short attack latencies had lower Cort levels than those selected for long attack latencies [62]. This links up with our findings, which show a direct positive correlation of attack latency with Cort, apparently a novel finding in the literature. These mice showing

higher levels of aggression may have an active behavioral stress response as mentioned earlier and thus, failure to mount an ACTH response may be the major reason for the low Cort levels [30,62].

4.4. Comparative study

The winner effect was highly significant in *P. californicus* males [7], but was not significant in the *P. leucopus* males, despite using the identical methodology and sample size. The species contrast may be attributable to the differences in aggression and territoriality. Note that many *P. leucopus* interactions appear to be resolved peacefully. As a result, a high percentage of *P. leucopus* males were removed from the winner effect study during training, while by comparison the percentage of males removed in the *P. californicus* study was miniscule.

There is a growing need to investigate qualitative aggression. Studies of attack topography suggest that the target of attack on an opponent's body may be used to indicate whether an attack is defensive or offensive [63,64], although there may be other reasons for target of attack such as what behaviors are used in dominant/subordinate interactions. In rodents, conspecific offensive aggression is thought to target the back and flanks of an opponent, while conspecific defensive aggression targets the face and snout of the opponent [64]. Most research has focused on investigating these forms of aggression within a single species [65–67], but the same approach applied to species differences may be equally illuminating. Interestingly, we found that the types of aggression displayed differed between these two species. *P. californicus* males tended to attack on the back and flank areas, whereas *P. leucopus* males tended to attack on the snout and face. This suggests that *P. californicus* males may show more “offensive” aggression while *P. leucopus* males may show more “defensive” aggression according to the definitions in use by other researchers [64]. A territorial, aggressive species that displays a clear winner effect (*P. californicus*) may be more likely to display more offensive aggression compared to a less aggressive species (*P. leucopus*) lacking a winner effect, which may display more defensive aggression. Hypothetically, these differences in qualitative aggressive behaviors could be related to the differential hormonal profiles found in each species. Based on this we can speculate that T may be more likely to mediate the display of offensive aggression [68,69], while Cort may be more likely to mediate the display of defensive aggression although this needs further investigation as there was a nonsignificant trend for lower Cort levels in *P. californicus* males expressing the winner effect compared to those of resident losers. Furthermore, it is possible that higher Cort levels may represent a type of depression as has been argued in other rodent species [70].

5. Summary

We found striking differences in the winner effect in two *Peromyscus* species. The effect is present in the species that is aggressive and territorial, *P. californicus*, and weak or absent in

P. leucopus, in which staged social interactions between males are much more peaceful. Other species differences may also be important because *P. californicus* males are also monogamous while expressing high levels of paternal care; while *P. leucopus* males are polygynous with very little paternal care. These species characteristics may help in understanding the clear winner effect in *P. californicus* males and its apparent weak effect in the *P. leucopus* males. The hormonal changes in association with winning may also play a role. A surge of T was associated with the winner effect in *P. californicus* males; whereas there was no significant change in T in *P. leucopus* males (at 45 min post-encounter). However, Cort was associated with winning in *P. leucopus*, and to a lesser nonsignificant level, in *P. californicus* males. These hormonal changes may be associated with rather different types of aggression or dominance. Further study is warranted to investigate whether T is associated more with offensive aggression and Cort more with defensive aggression.

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