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# Winning fights elevates testosterone levels in California mice and enhances future ability to win fights

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## Abstract

The ‘winner effect’ has been studied in a variety of species, but only rarely in mammals. We compared effects of winning three, two, one, or zero resident–intruder encounters on the likelihood of winning a subsequent aggressive encounter in the California mouse (*Peromyscus californicus*). During the training phase, we ensured that resident males won all encounters by staging contests with mildly sedated, smaller intruders. During the test phase, the resident male encountered an unfamiliar, more evenly matched intruder that had experience winning an encounter and was larger than the resident. Testosterone (T) plasma levels significantly increased after the final test when they had experienced two prior winning encounters, and the probability of winning a future encounter increased significantly after three prior wins independent of intrinsic fighting ability. We hypothesize a ‘winner–challenge’ effect in which increased T levels serve to reinforce the winner effect in male California mice.

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*Keywords:* Winner effect; Aggression; Testosterone; *Peromyscus californicus*; Rodent; Mammal; Challenge hypothesis

## Introduction

The winner effect, an increased probability of winning an aggressive encounter following previous victories (Dugatkin, 1997), has been demonstrated in a variety of nonmammalian species (fish: Hsu and Wolf, 1999; review of winner effect in fish, Chase et al., 1994; reptiles: Schuett, 1997; and insects: Moore et al., 1988; Otronen, 1990; Whitehouse, 1997). The winner effect can overcome a large male advantage (Whitehouse, 1997) and in some cases is as important as the loser effect in an ensuing encounter (Hsu and Wolf, 1999). There are species differences in the occurrence and relative importance of the winner effect. Although some of these apparent differences may have a methodological basis such as nonrandom assignment of individuals to ‘winning’ and ‘losing’

categories and length of time between aggressive encounters (discussed by Chase et al., 1994), some species differences are likely related to the costs and benefits of fighting, including the risk of injury and the probability of aggressive social interactions occurring (Hsu and Wolf, 1999; Schuett, 1997). It is also likely that the social system, especially the incidence of territoriality, has a significant effect on winning behavior.

Although the winner effect has yet to be clearly demonstrated in mammals while controlling for intrinsic abilities, there is substantial evidence that a single aggressive encounter can increase future aggression, regardless of whether a clear winner has been identified (e.g., Bevan et al., 1960; Martinez et al., 1994; Parmigiani and Brain, 1983; Potegal and Coombes, 1995; Trainor and Marler, 2001). This increased aggression can take the form of a shorter attack latency, increased frequency of initiated attacks, or increased duration of aggression (Kudryavtseva, 2000; Martinez et al., 1994; Parmigiani and Brain, 1983). While it is quite plausible that increased aggression after aggressive interactions or a single winning experience may

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translate into an increased likelihood of winning a future aggressive encounter, this has not specifically been tested in mammals. The closest suggestion of a mammalian winner effect was studied using a dominance hierarchy and found that dominant mice are more likely to defeat subordinates (Burg and Slotnick, 1982); however, a demonstration of the winner effect disregarding intrinsic fighting ability has not been tested. Here, we ask whether prior winning experience can also lead to future winning in mammals, specifically, in the very aggressive and territorial California mouse, *Peromyscus californicus*, that defends territories year-round (Ribble, 1990).

Additionally, a mechanism(s) for the winner effect needs to be demonstrated, although a number of possibilities exist. Repeated wins may increase future winning ability because winners become better fighters possibly by learning how to win more efficiently such as by using better strategies (Hsu and Wolf, 2001). There may also be variation in the motivation to initiate an encounter, escalate an encounter, or more rapidly attack an individual. A variety of physiological mechanisms could contribute to this phenomenon, including neural changes involving learning and hormonal changes such as testosterone increases (Hsu and Wolf, 2001). Another aspect of aggressive behavior, generally treated independently of the winner effect, is addressed as the ‘Challenge Hypothesis,’ which posits an increase in testosterone (T) after a single aggressive encounter (Wingfield et al., 1987). This occurs across a wide variety of species (reviews by Cavigelli and Pereira, 2000; Oliveira et al., 2001; Yang and Wilczynski, 2002). Human studies have also investigated T changes after a variety of social challenges (review by Mazur and Booth, 1998). For example, T levels increase in the winners of a competitive encounter such as a tennis match (Booth et al., 1989) as well as in spectators supporting a winning team (Bernhardt et al., 1998). Despite the substantial literature on the Challenge Hypothesis, the function of transient increases in T after a win remains unclear. It has been suggested that T may function to sustain, but not initiate, appropriate aggressive responses during and after an aggressive encounter (Wingfield et al., 1987).

One of our goals was to begin integrating the winner effect and the challenge hypothesis (The ‘Winner–Challenge Effect’). We hypothesize that the changes in circulating T may have a direct influence on the ability to win future aggressive encounters. We had predicted that post-winning T levels were especially likely to increase after an encounter in *P. californicus* because it is a territorial species and because baseline T levels might be predicted to be lower in males of species that display paternal behavior (Wingfield et al., 2000; although see Marler et al., 2003 for complications of this hypothesis for *P. californicus*). In addition, male *P. californicus* mice have lower baseline T levels than other *Peromyscus* species, (Marler et al., 2003) and thus, perhaps a more readily measurable increase in T levels after an initial aggressive interaction, as their

baseline T levels may not be at their maximum (Beletsky et al., 1995). A study conducted in parallel offers further support for an association between T and the winner effect because transient increases in T increased future aggression in California mice (Trainor et al., 2004). Winning without a transient increase in testosterone (castrated males given baseline T-implants) did not cause an increase in future aggression. Here, we examine whether a winner effect and/or challenge effect occurs in male California mice.

To explore the winner effect, we investigated how winning zero to three resident–intruder encounters affected the likelihood of winning a final, more evenly matched aggressive encounter in *P. californicus* males. We used a random selection paradigm (Chase et al., 1994) to control for intrinsic fighting ability and prior dominance ranking while group housed. We ensured that resident males won all initial training encounters by using mildly sedated small males as intruders. For the final aggressive encounter, we arranged for the resident male and the unfamiliar intruder to be fairly evenly matched. In addition, hormonal changes were examined after the final winning encounter. We measured T and used Corticosterone (Cort) as a hormonal control for the stress of blood sampling, as Cort has been associated with stress in *Peromyscus* species (Demas and Nelson, 1998).

## Methods

### Subjects

Sexually naïve male *P. californicus* 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 14L:10D 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 (48 × 27 × 16 cm) and fed Purina 5001 mouse chow and water ad libitum. Because males were housed in groups, the subjects most likely experienced fighting or dominance interactions; however, animals were randomly assigned to groups, therefore controlling for any prior experience. All of the mice in the experiment ranged in age from 5.5 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 moved into experimental rooms that were kept under a 14L:10D 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 132 male mice was used, of which 50 were test subjects, another 50 were final intruders during the testing phase, and 32 were intruders during the training phase, as described below.

## Behavioral testing

### General procedures

Forty *P. californicus* males were randomly assigned to win either zero, one, two, or three times during the training phase ( $n = 10$ /condition, see Table 1). An additional ten males were randomly assigned to a control group that experienced no training or test encounters and served as a hormonal control (see Hormonal measurements section). In the training phase (see below), each of the forty males was exposed to either an aggressive encounter or a control experience for a total of three training experiences (Table 1). 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).

On Day 1, subjects were weighed (mean  $\pm$  SE: 42.3  $\pm$  0.89 g) and paired with a female. To ease identification and removal, a small area of the female's back was shaved (see Trainor et al., 2004). On Day 11, each sexually experienced male and its mate were placed in a large glass observation cage. The observation cage (60  $\times$  30  $\times$  30 cm) contained a small nest box (17.5  $\times$  17.5  $\times$  17.5 cm), a plastic tube for enrichment (length 22 cm, radius 5 cm), Purina 5001 mouse chow, and a water bottle. The nest box was fully enclosed except for a small opening (5 cm diameter) in the lower corner for passage into it.

All aggressive trials (Days 13, 15, 17, and 19) followed the resident–intruder paradigm (Bester-Meredith et al., 1999; Trainor and Marler, 2001), 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 before each trial. At the beginning of the trial, an opaque divider was inserted to separate the resident from the intruder and the intruder was introduced into the cage. The resident and intruder were separated for 2 min to allow the intruder to acclimatize to the cage. The divider ensured that the resident was on the side containing the nest box. After the divider was removed, we videotaped the resident and intruder interacting for 10 min for all encounters. A single observer, blind to treatment groups, recorded the frequencies for each individual of biting, chasing, wrestling bouts (a stint of wrestling that lasts at least 3 s), jumping away,

and retreating, as well as the total time spent freezing. Freezing behavior was defined as remaining in a fixed position after a fight (Scholtens et al., 1988). The winner/loser status was established when at least three attacks occurred during the trial, consistently initiated by the same individual and eliciting avoidance or freezing behavior from the opponent. Based on Eisenberg's (1961) studies of *Peromyscus* behavior, winning behavior was operationally defined as initiating attacks of biting, chasing, and wrestling behavior, while losing behavior included jumping away (from the opponent), freezing, and retreating (see also Kudryavtseva, 2000). 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 3 of 43 training sessions and these trials were excluded from the study. No encounter resulted in obvious injury to the participants.

Attack latency of the winner and freeze latency of the loser were also recorded. Attack latency was measured as the time (0–600 s) at which the resident first attacks (bites or wrestles) the intruder. Freeze latency is a measure of how quickly the losers exhibited losing behavior and was measured as the time (0–600 s) at which the intruder first displays freezing behavior.

### Training phase

To increase the probability that residents had only winning experiences during the training phase, encounters with unfamiliar opponents were biased in the following way. Each individual selected to be a winner was a resident, larger than the intruder (mean difference  $\pm$  SE: 8.3  $\pm$  1.1 g), sexually experienced, and fully alert. The resident advantage (see Bester-Meredith et al., 1999; Marler et al., 2003; Trainor and Marler, 2001) and sexual experience (Wang et al., 1997) are both associated with increased aggression. On the other hand, opponents during the training phase had the disadvantage of being intruders, smaller, sexually inexperienced, mildly sedated, and former losers of 1–2 aggressive encounters prior to the experiment. To normalize individual intruder differences between encounters without increasing injury to the intruder during the attack, intruders were mildly sedated with a 5 mg/kg intraperitoneal injection of the non-narcotic analgesic methotrimeprazine (Potegal et al., 1980). The treated animals were still capable of eliciting an attack from the resident and responding to the attack (Potegal and Coombes, 1995; Potegal et al., 1980). Intruders in the training phase were weighed 3 days before the encounter to reduce the amount of disturbance involved immediately before the aggressive encounter. These intruders were injected and placed in an unfamiliar cage in isolation for 10 min in order to allow enough time for the methotrimeprazine to take effect before being introduced into the resident's cage. This sedation appeared to reduce locomotion and offensive aggression without reducing defensive aggression or losing behavior (see also Potegal and Coombes, 1995). Each intruder was randomly assigned

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	Handle	Handle	Handle	Handle
Condition 1	Handle	Handle	Handle	Test
Condition 2	Handle	Handle	Win	Test
Condition 3	Handle	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.

to its resident opponent and was used no more than twice, each time with different opponents.

#### *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  $\pm$  SE:  $5.7 \pm 0.9$  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 final intruder was randomly assigned to its resident opponent and was used only once.

#### *Hormonal measurements*

Baseline blood samples were taken from the 50 males that were randomly selected to be winners and controls 1 week before the training phase during the early part of the dark cycle (between 1330 CST and 1530 CST) to estimate baseline hormone levels. This blood sample was obtained from the retro-orbital sinus within 2 min (mean  $\pm$  SE:  $48.3 \pm 3.7$ s) of removing the male from the cage. Forty-five minutes after the final test encounter, trunk blood (a second blood sample) was collected by rapid decapitation. The 45-min interval was chosen 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 (Sachser and Prove, 1984) and a timeline for T changes (at 0, 15, 30, 45, 60, 90, and 1440 min post-encounter) suggests that T increases at 45 min after an aggressive encounter (Marler et al., 2005). All samples were immediately centrifuged to obtain the plasma and stored at  $-80^{\circ}\text{C}$  until assayed. Hormone assays were carried out at the Wisconsin Primate Research Center. Samples were extracted with ethyl ether and steroids were separated using celite chromatography. 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 using a method previously validated for California mice and described elsewhere (Bester-Meredith and Marler, 2001; Trainor and Marler, 2001). For T levels, the intra- and inter-assay coefficients of variation were 8.2% and 17.0%, respectively ( $n = 3$  plates). For Cort levels, the intra- and inter-assay coefficients of variation were 5.8 and 13%, respectively ( $n = 4$  assays).

#### *Statistical analyses*

All statistical analyses were performed using Statistica Version 6, Statsoft, Inc., Tulsa, OK and SPSS Version 11.0. Attack latency and freeze latency were log transformed as  $Q-Q$  plots indicated that this transformation rendered the

most normally distributed data (Zar, 1996). Frequency of bites, chases, and wrestling bouts were analyzed separately and together as total aggressive behavior. Aggressive behavior was combined because the frequency and duration of each behavior varied considerably by individual, such that separate behaviors were less likely to reach significance (also see Scholtens et al., 1988). Avoidance behaviors, however, were analyzed separately as the mode of data collection (duration vs. frequency) differed among the three behaviors (jumping, retreating, freezing), as described earlier. The winner effect was analyzed using Fisher's exact test. One-way ANOVA,  $t$  tests, Fisher LSD post hoc tests, and Pearson's correlations were used to analyze all other behavioral data.

Testosterone and Cort data were log transformed for all statistical analyses as  $Q-Q$  plots revealed that this transformation provided the most normally distributed data. We always retained baseline hormonal levels (T or Cort) and assay number as covariates in the model to explain variation in the data. Possible covariates retained in the models of hormonal analyses were time to obtain blood sample and resident age and are reported in the results. Across all T assays in winners and controls, a total of three blood sample values out of 70 was off the standard curve and was not included in the analysis (one sample was removed from conditions 2, 3, and the control condition). No blood sample values were off the standard curve in the analysis of Cort levels. One-way ANCOVA, general regression, and Fisher LSD post hoc tests were used to analyze all hormonal data.

Apart from the winner effect analyses, all other analyses focused on resident winners of the final encounter only. With these methods, we could not ascertain if resident losers perceived themselves as losers, because they had the opportunity to reestablish their territory for 45 min before the final blood sample was taken. The design we used is not typical for creating 'losers' (see methods for creating losers: Huhman et al., 1991; Sachser and Lick, 1991). Because of these methodological limitations, T levels from losers of the final encounter were not analyzed.

## **Results**

### *The winner effect*

The experience of winning increased the probability of winning the final encounter in *P. californicus* males ( $\chi^2 = 11.4$ ,  $df = 3$ ,  $P = 0.01$ , Fig. 1), but only males with three previous winning experiences differed significantly from males with no previous experience ( $P < 0.001$ ). Cumulative changes may have been revealed using larger sample sizes.

### *Behavioral data (resident winners)*

A one-way ANOVA revealed that resident attack latency decreased significantly with the number of previous wins

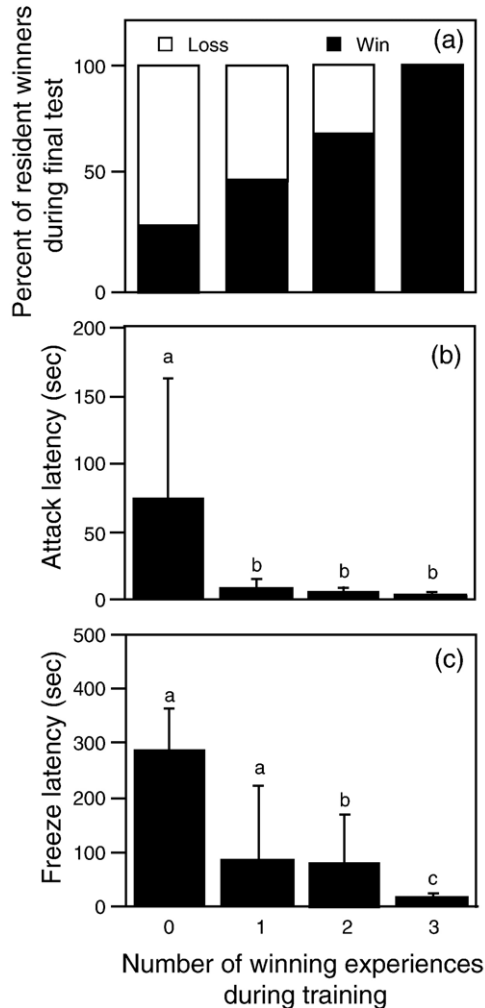


Fig. 1. The winner effect. (a) The percentage of males that won the final encounter across the four conditions ( $n = 10$  per condition). (b) Attack latency during the final encounter across the four conditions of prior winning experience. (c) Freeze latency of the final intruders across the four conditions of prior winning experience. Groups that share the same letter are not significantly different from each other ( $P > 0.05$ ). Data in panels b and c are presented as means  $\pm$  standard errors.

[ $F(3,22) = 4.72$ ,  $P = 0.011$ , Fig. 1]. A Fisher LSD post hoc test showed that males with no previous winning experience had significantly longer attack latencies during the final encounter than males with one ( $P = 0.018$ ), two ( $P = 0.002$ ), or three ( $P = 0.003$ ) previous winning experiences. In contrast to the data on the probability of winning, there appeared to be no cumulative effect of prior winning experience; attack latency decreased after one win, but did not decrease further with additional wins [ $F(2,20) = 0.743$ ,  $P = 0.488$ ].

The freeze latency of the intruder in the final encounters decreased significantly with the number of previous wins by the resident male [ $F(3,22) = 5.05$ ,  $P = 0.008$ , Fig. 1]. A Fisher LSD post hoc test showed that, compared to interactions with resident males who had no previous winning experience, intruders froze earlier when interacting

with males who had two ( $P = 0.032$ ) and three ( $P = 0.001$ ) previous winning experiences, with a nonsignificant trend for males with one previous winning experience ( $P = 0.069$ ). There was also evidence for a cumulative effect of a resident's prior winning experience on the behavior of intruders; the intruder froze earlier when interacting with a male with three previous winning experiences compared to males with two ( $P = 0.041$ ) and one ( $P = 0.045$ ) previous winning experiences. This indicates that the intruders in the final encounter, which all had equal prior experience, exhibited losing behavior sooner as the amount of previous winning experience of the resident increased. Overall, attack latency by the resident and freezing behavior by the intruder differed because only freezing behavior by the intruder exhibits a cumulative effect of experience that was statistically significant.

The amount of prior winning experience did not significantly alter any other individual aggressive behaviors observed during the aggressive interactions (all  $P > 0.2$ ); however, total aggressive behavior in the resident winners (total bites, chases, and wrestling bouts) decreased with increasing previous winning experience [ $F(3,22) = 3.09$ ,  $P = 0.048$ ]. Similarly, total aggressive behavior was also positively correlated with attack latency ( $r = 0.44$ ,  $P = 0.006$ ,  $n = 26$ ) and freeze latency in intruders ( $r = 0.55$ ,  $P < 0.001$ ,  $n = 26$ ) (Fig. 2). In addition, intruder freeze latency from the first resident attack decreased significantly with increasing prior winning experience [ $F(3,22) = 3.57$ ,  $P = 0.023$ ]. This suggests that, with more experience, the resident was able to elicit loser behavior from an intruder more quickly.

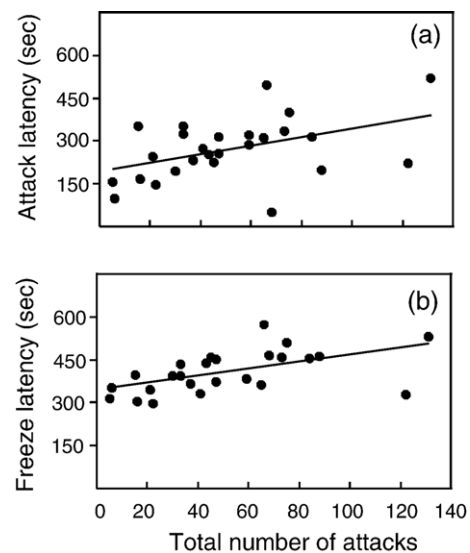


Fig. 2. (a) Relationship between attack latency of the resident and total aggressive behavior during the final encounter in resident winners only. (b) Relationship between freeze latency of the intruder and total aggressive behavior of the resident during the final encounter in resident winners only.

Overall, the behaviors were significantly related to the result of the encounters such that winners (residents) displayed more total aggressive behavior than the losing intruders and the losing intruders froze, jumped away, and retreated more than the winners [Total aggressive behavior,  $F(1,38) = 17.91$ ,  $P < 0.001$ ; Freezing,  $F(1,38) = 25.94$ ,  $P < 0.001$ ; Jumping Away,  $F(1,38) = 29.56$ ,  $P < 0.001$ ; Retreating,  $F(1,38) = 7.37$ ,  $P = 0.009$ ].

#### Hormonal data (resident winners)

##### Cort control

Cort levels were not different after winning when compared to controls (Figs. 3 and 4), with no relationship with increasing prior winning experience. Levels were within the range found in other studies of California mice (e.g., Glasper and DeVries, 2005).

##### Testosterone levels

The model that best explained the variation in T levels [adjusted  $r^2 = 0.606$ ;  $F(8,21) = 6.57$ ,  $P < 0.001$ ] incorporated time to obtain blood samples [ $F(1,21) = 7.31$ ,  $P = 0.314$ ] and resident age [ $F(1,21) = 2.75$ ,  $P = 0.112$ ] as covariates. Analyses revealed a challenge effect; T levels in winners of the final encounter were higher than those of controls using a one-way ANCOVA [ $F(1,24) = 4.74$ ,  $P = 0.035$ , Fig. 3].

Increases in T levels, after the final encounter, with prior winning experience were similar to the patterning of the winner effect. A one-way ANCOVA detected a significant increase in the T levels of winners of the final encounter as prior winning experience increased [ $F(4,21) = 3.70$ ,  $P = 0.019$ , Fig. 4]. An LSD Fisher test indicated that the average T level in individuals with three previous winning experiences was significantly higher than that of controls ( $P = 0.006$ ), and individuals with one ( $P = 0.031$ ) previous winning experience or none ( $P = 0.021$ ), but did not differ from the T level of individuals with two previous winning experiences. Fisher LSD post hoc tests revealed that T levels of individuals with two previous winning experiences were significantly higher than controls ( $P = 0.044$ ), but were not

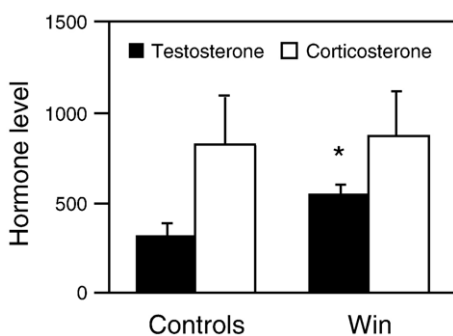


Fig. 3. Testosterone (pg/ml) and Cort (ng/ml) levels in controls and all resident winners. Testosterone levels increase with prior winning experience. Data are presented as means  $\pm$  standard errors. \* $P < 0.05$ .

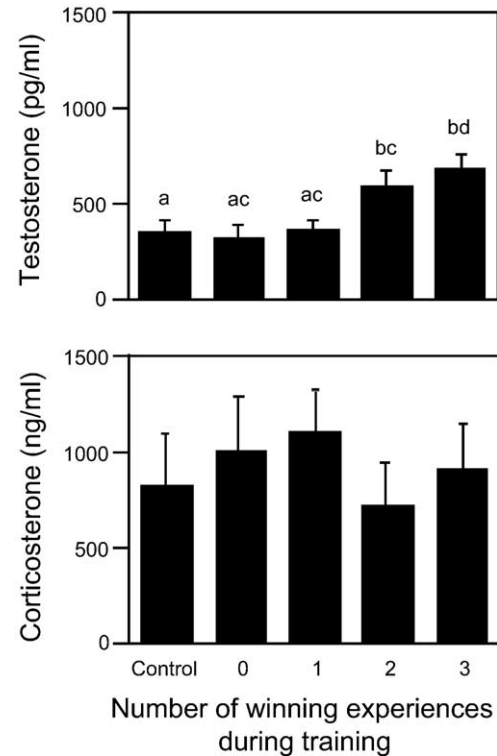


Fig. 4. Testosterone and Cort levels across the five conditions. Testosterone increases significantly in males that win more than two previous encounters compared to controls, and in male winners that win three previous encounters compared to male winners that have won zero previous encounters. In contrast, Cort levels remain the same regardless of winning experience. Groups that share the same letter are not significantly different from each other ( $P > 0.05$ ). Data are presented as means  $\pm$  standard errors.

significantly different from males with no or one prior winning experience.

## Discussion

The data demonstrate a winner effect in *P. californicus*, i.e., winning multiple prior encounters increases a male's ability to win future encounters regardless of his intrinsic fighting ability. On a physiological level, we also have evidence that endogenous T levels change in response to experiencing a winning encounter. The winner effect and the challenge effect can therefore occur in a highly territorial mammalian species.

### Behavior

In *P. californicus* males, the winner effect was detected statistically only after winning three previous encounters. Individual winning encounters appear to have long-lasting, cumulative effects that transfer to subsequent aggressive encounters to alter future winning behavior. Previous research in fish has indicated that the winner effect can last anywhere from seconds (Chase et al., 1994) to 3 days (Hollis et al., 1995). The present data on *P. californicus*

indicate that the effect of winning an encounter on future winning behavior lasts at least 2 days. Because it was necessary to have three previous winning encounters (each 2 days apart) to significantly influence ability to win, the winner effect may even last more than 6 days in *P. californicus*.

The late emergence of the winner effect indicates that future behavior may not be modified strongly until an individual has sufficiently sampled the social environment and gained adequate experience. [Beaugrand and Goulet \(2000\)](#) suggest that prior winning experience may be more quantitative rather than qualitative. Hence, the number of previous winning encounters an individual has experienced may be more important for establishing the winner effect than the aggressive behavior occurring during a single encounter. Our study supports the idea that quantitatively, the number of previous wins is critical to achieve the winner effect, but that qualitatively aggressive behaviors such as attack latency may still be important. Moreover, [Hsu and Wolf \(2001\)](#) suggest that study of the behavioral strategies of the resident during each encounter may be necessary to distinguish between outcomes of these aggressive encounters. Hence, in addition to investigating the winner effect, we also studied how resident and intruder behaviors (we do not regard intruder behaviors as part of the behavioral strategy, but rather as an indirect measure of what the resident is communicating) are altered in response to winning. Attack and freeze latencies both decreased with increasing winning experience, suggesting that both could contribute to the winner effect. Serial aggressive encounters, without winning experience per se, have a similar effect on attack latency ([Potegal and Coombes, 1995](#)).

There was also a significant relationship between attack latency, freeze latency, and total aggressive behavior. Males that attacked earlier displayed less aggressive behavior overall, than those that attacked later. Even though this result appears counterintuitive at first glance, several studies have obtained similar findings in encounters with an unfamiliar intruder ([Kudryavtseva, 2000](#); [Parmigiani and Brain, 1983](#)). Moreover, a greater amount of total aggressive behavior by the resident was associated with a longer freeze latency by the intruder. Thus, shorter attack latencies and less aggressive behavior by the resident were associated with more rapid displays of submissive behavior by the intruder. It is conceivable that the relationship between attack latency, freeze latency, and aggression changes with repeated winning experiences, such that males that attack earlier (attack latency) win faster, by causing intruders to freeze sooner (freeze latency), thereby alleviating the need to display more aggressive behavior. No other individual behaviors, such as jumping away, retreating, and duration of freezing, played a detectable role in the winner effect. We infer that there must be some unmeasured form of perhaps learned visual, auditory, or chemical communication (e.g., postures, vocalizations, or pheromones) that causes intruders to freeze sooner in these circumstances in spite

of fewer overt aggressive behaviors from the resident. Without further testing, it is unknown whether repeated exposure to different males' odors or repeated experiences of increased arousal or activity could cause the winner effect. It is also plausible that winning may serve as a reward or reinforcer ([Legrand, 1978](#)); however, the experience of winning alone is unlikely to increase aggression, as winning without changes in T levels (but maintaining a baseline level of T) does not result in increased aggression in future encounters ([Trainor et al., 2004](#)). Thus, activity alone or arousal independent of changes in T does not appear to cause changes in future aggressive behavior within male California mice.

### Hormones

The challenge hypothesis, in which T increases after an aggressive challenge ([Wingfield et al., 1990](#)), was supported by our data on *P. californicus* mice. Individuals that won the final encounter had significantly higher T levels than those of controls, as found in several other mammals (e.g., rodents: [Buck and Barnes, 2003](#); [Sachser and Prove, 1984](#); non-human primates: [Cavigelli and Pereira, 2000](#); humans: [Mazur and Booth, 1998](#)). The challenge effect may vary with species and level of aggressiveness because house mice selected for low aggressiveness and high emotionality, CBA/Lac, do not display the challenge effect ([Kudryavtseva et al., 2004](#)). Not only was the challenge effect observed, T also paralleled the emergence of the winner effect, as the T levels of resident winners increased with prior winning experience. Specifically, among individual groups, the increase in T was only identified in residents with two previous winning encounters, and the winner effect was not statistically detectable, compared to males with no previous wins, until after winning three previous encounters. While this may be influenced by the small sample size for winners in groups with fewer winning encounters, it is consistent with our hypothesis that the T surge following an encounter can serve to increase the likelihood of winning future aggressive encounters. This conclusion is further supported by the finding that while intact *P. californicus* males with previous winning experience in resident–intruder aggression tests exhibited shorter attack latencies in subsequent tests, castrated males showed no such change in attack latencies with previous experience ([Trainor and Marler, 2001](#)) and aggression does not increase as a result of winning experiences alone without transient increases in testosterone ([Trainor et al., 2004](#)). [Wingfield et al. \(1987\)](#) suggested that the function of the increase in T may be to sustain appropriate aggressive behavior during an encounter. Other investigators have also suggested other factors such as emotions may sustain behavior (i.e., frustration-related aggression) as it relates to timing of rewards ([David et al., 2004](#)). Our results are consistent with our extension of [Wingfield's](#) hypothesis proposing that T serves to sustain winning behavior during future aggressive encounters as



well. Thus, the data are consistent with a ‘winner–challenge effect’ phenomenon that a transient increase in T after an encounter functions to increase future ability to win an encounter.

### *The winner–challenge effect*

In summary, we have novel data suggesting that prior winning experience contributes positively to the likelihood of winning future aggressive interactions in male *P. californicus* mice. To our knowledge, this, in combination with the results of Trainor et al., 2004, provides the first association between the winner effect (repeated winning experiences) and changes in T levels in a mammalian species. We hypothesize that T causes changes in future behavior via longer-term modification of related neurochemical systems such as arginine vasopressin (Ferris and Delville, 1994), serotonin (Summers et al., 2000), or dopamine (Kudryavtseva, 2000), known to be associated with aggression, and hence, perhaps directly influenced by testosterone.

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