# How to Design Experiments in Animal Behaviour*

## 11. Fighting Fish—Does Experience Matter?

*Raghavendra Gadagkar*

**Wonderful as they are, insects do not by any means exhaust the possibilities of suitable organisms to conduct fascinating, cutting-edge, low-cost research, especially in animal behavior. Having seen how insects can be used to this end, in all the previous articles in this series, I will now deliberately choose examples from studies done on vertebrates, starting with fish and navigating through the evolutionary tree of life, all the way to humans. In this article, we will see how simple, clever experiments can reveal that when fish fight, the outcome is not only based on their intrinsic fighting abilities but also on extrinsic factors such as prior winning and losing experiences, and indeed, on a sophisticated interaction between intrinsic and extrinsic factors. In particular, we will study the phenomenon of winner-effects and loser-effects and learn that this is a near-virgin field of research waiting to be exploited and eminently suitable for cutting-edge research at a trifling cost.**

**Raghavendra Gadagkar is DST Year of Science Chair Professor at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore, Honorary Professor at JNCASR, and Non-Resident Permanent Fellow of the Wissenschaftskolleg (Institute for Advanced Study), Berlin. During the past 40 years he has established an active school of research in the area of animal behaviour, ecology and evolution. The origin and evolution of cooperation in animals, especially in social insects, such as ants, bees and wasps, is a major goal of his research.
http://ces.iisc.ac.in/hpg/ragh.
https://www.researchgate.net/profile/Raghavendra_Gadagkar**

### Fighting Again, But This Time in Fish

Insects were the protagonists of all the previous ten articles in this series. But, as I said in the previous article [1], I do not want to leave you with the impression that such experiments can only be done with insects. As promised, we will now consider the design of similarly simple and low-cost experiments that can be performed with equal ease with higher animals namely, vertebrates. We will begin with fishes in this article and navigate through the evolutionary tree of life, all the way to humans, in subsequent ar-

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ticles. Here, we will focus on fighting in fish. Readers of this series may begin to get a bit wary of my apparent obsession with fighting in animals. I promise to make an attempt to focus on behaviours not involving fighting in some of the future articles. But I must say in my defense, that fighting is a ubiquitous and important component of the social life of animals. Moreover, we will see in this article that a little bit of fighting helps to reduce much subsequent fighting. This is because animals seem to gain experience during fights and use that experience to decide whether or not to indulge in subsequent fights.

As is surely true in humans, in a variety of animal species too, individuals seem to gain confidence if they win a fight and lose confidence if they lose a fight. This can be inferred from the observation that winners of a fight are more likely to win their next fight and losers of a fight are more likely to lose their next fight. These phenomena are referred to as winner-effects and loser-effects.

As is surely true in humans, in a variety of animal species too, individuals seem to gain confidence if they win a fight and lose confidence if they lose a fight. This can be inferred from the observation that winners of a fight are more likely to win their next fight and losers of a fight are more likely to lose their next fight. These phenomena are referred to as winner-effects and loser-effects. In other words, experience gained in fighting, whether it be a winning experience or a losing experience, plays a significant role in future fighting behavior, fighting decisions and fighting outcomes. We can design simple low-cost experiments to detect the presence or absence of winner- and loser-effects in different animal species. It turns out that fish species have been a favourite choice for such experiments, and we will see several examples. While these experiments may indeed require little by way of facilities and equipment, they nevertheless, need great care in designing the experiments lest we draw erroneous conclusions, and that is another reason why I have chosen to illustrate this topic.

## Winner-Loser Effects

### *Experimental Design*

#### *Self-selection*

To detect winner- and loser-effects, we first stage a contest between two randomly chosen individuals, identify the winner and loser and separate them. To do this, it is best to pair individuals as closely matched as possible in their fighting abilities, based on body size, age or whatever we know about the species. This is especially useful if our sample sizes are likely to be small. Then we can stage a second contest separately for both the winner and the loser, with other randomly chosen individuals who have not had any recent winning or losing experience. If the winner in the 1st contest wins the 2nd contest more often than expected by chance alone, then we have discovered a winner effect. Similarly, if the loser in the 1st contest loses the second contest more often than expected by chance alone, we have discovered a loser effect. But what is expected by chance alone? Many investigators have assumed that winners and losers in the first contest have equal probabilities of winning or losing the 2nd contest, in the absence of winner/loser effect, i.e, by chance alone. Thus, they have inferred winner and loser effects when winners and losers in the 1st contest have won and lost their 2nd contest respectively with a probability that is significantly greater than 50%.

If the winner in the 1st contest wins the 2nd contest more often than expected by chance alone, then we have discovered a winner effect. Similarly, if the loser in the 1st contest loses the second contest more often than expected by chance alone, we have discovered a loser effect.

Let us now describe this more formally. Let us represent a win by W and a loss by L. At the end of the first contest, we will have identified winners and losers which we can label as W and L respectively, based on their respective fighting histories. At the end of the second contest, we will have individuals which we can label as WW and WL, based on their two fighting histories, when we are trying to find a winner effect. The null hypothesis has often been assumed to be that the frequency of WW = the frequency of WL, so that if the frequency of WW was significantly greater than the frequency of WL, then a winner effect has been inferred. Similarly, in an attempt to detect a loser effect, the null hypothesis has been assumed to be that the frequency of LW = the frequency of LL and hence, if the frequency of LL was significantly greater than the frequency of LW, then a loser effect has been inferred.

Unfortunately, the null hypothesis of 0.5, assumed by many researchers is wrong. In this experimental design, the experimenter does not decide who will win and who will lose the 1st contest. Hence, winners and losers entering the 2nd contest have not been randomly chosen. The contestants that entered the 1st contest were chosen randomly, but the experimenters had no control over who will become the winner and who will become the loser. This was decided by the animals themselves. Hence this experimental design is called 'self-selection'—here, the 'self' refers to the animals and not to the experimenters. The problem with such self-selection is that even though the contestants for the 1st contest were chosen randomly, the winners and losers are not random individuals, i.e., they are not drawn randomly from the distribution of fighting abilities in the population. It is reasonable to assume that the winners in the 1st contest are among those that have relatively better fighting abilities and the losers in the 1st contest are among those with relatively poorer fighting abilities. As a consequence, winners in the 1st contest have a better than even chance of winning the 2nd contest with a random individual, and losers in the 1st contest have a greater than even chance of losing the 2nd contest with a random individual. It has been shown mathematically by Bégin *et.al.*, (1996) [2] that because of such self-selection, winners and losers in the 1st contest have a 2/3rd probability of winning and losing their second contests, respectively. Hence the null expectation should be 67% and only if WW has a significantly higher probability than 0.67 and LL has a significantly higher probability than 0.67, should we infer winner- and loser-effects.

It is easy enough to re-do the statistics for already published studies and confirm whether winner- and loser-effects were erroneously inferred or whether they are still valid under the new criterion, and that is what Bégin *et. al.*, (1996) [2] have done for some studies published prior to their 1996 paper in which they also mathematically proved the appropriateness of the 0.67 criterion and the inappropriateness of the 0.5 criterion.

It is easy enough to re-do the statistics for already published studies and confirm whether winner- and loser-effects were erroneously inferred or whether they are still valid under the new criterion, and that is what Bégin *et.al.*, (1996) [2] have done for some studies published prior to their 1996 paper in which they also mathematically proved the appropriateness of the 0.67 criterion and the inappropriateness of the 0.5 criterion. They examined five previously published studies and found that four out of five studies, including two of their own studies, had wrongly inferred winner/loser effects using the 0.5 criterion, and their results did not satisfy the 0.67 criterion; only one of the five studies they tested (fortunately their own!) held up in spite of the 0.67 criterion. As Bégin *et.al.*, (1996) emphasize, self-selection is not wrong but the null hypothesis of equiprobability of WW and WL in the 2nd contest, in the case of winner effects, and the equiprobability of LL and LW in the 2nd contest in the case of loser-effects is not valid. Instead, the outcome of the 2nd contest should be judged against a null hypothesis of 2/3rds probability of WW or LL, for inferring winner- and loser-effects, respectively.

*Random-selection*

Although self-selection seems easy and the most natural way to select winners and losers (in the 1st contest) to test for winner and loser effects (in the 2nd contest), there is another way to design the experiment. This is called 'random-selection'. Here, we pick a random member of the population and ensure that it has a winning or losing experience, as per our choice (see below). Now we pair such randomly chosen animals with the experience of winning or losing, with other randomly chosen individuals that have no recent experience of winning or losing. If we do this with several randomly chosen individuals, it is likely that both winners and losers (in the 1st contest) will indeed be randomly distributed in their fighting abilities and hence, they will be expected to win or lose their 2nd contest with an equal probability, if there are no winner or loser effects. Thus, our null expectation now will legitimately be 0.5 and therefore, if WW is significantly more probable than WL, we can infer a winner effect and similarly, if LL is significantly more probable than LW, we can infer a loser effect.

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But how do we ensure that our randomly chosen individual has a winning or losing experience at our will? This can be done in different ways depending on the species being studied. In some species fighting ability is strongly linked to body size and/or age of the individuals. In such cases, any randomly chosen individual can be paired with another individual who is smaller and/or younger than itself so that the randomly chosen individual is sure to win. If many randomly chosen individuals are thus paired with partners who are smaller and or/younger than them, we can obtain a number of individuals with a winning experience who themselves span the whole distribution of fighting abilities. Now, when these winners (in the 1st contest) are paired with randomly chosen individuals with no winning or losing experience, we can separate the effect of intrinsic fighting ability and the effect of experience. With such a 'random-selection' procedure, we can set a cut-off of 0.5 in the 2nd contest. Thus, we can infer a winner effect if WW occurs significantly more than WL and a loser effect if LL occurs significantly more often than LW. In many species of fish, residents (individuals in their territory) are more likely to win fights with intruders (individual outside their own territory and the opponent's territory). Hence, we can also pair randomly chosen individuals in their own territory with intruders and ensure that the randomly chosen individual has a winning experience. Conversely, we can pair our randomly chosen individual with an opponent in the opponent's territory to ensure that the randomly chosen individual has a losing experience. Of course, it is best to stage the final contest in neutral territory.

In some species, there may not be any obvious external markers of fighting ability such as body size or age. Nevertheless, there may well be internal correlates of fighting ability such as hormone levels, or, what is more generally called 'aggressiveness' which includes a willingness to initiate a fight or escalate an already initiated fight.

In some species, there may not be any obvious external markers of fighting ability such as body size or age. Nevertheless, there may well be internal correlates of fighting ability such as hormone levels, or, what is more generally called 'aggressiveness' which includes a willingness to initiate a fight or escalate an already initiated fight. There is a clever way to deal with this situation. We need to pair randomly chosen individuals with habitual losers so that we can guarantee that our randomly chosen animal has a winning experience. Similarly, we need to pair our randomly chosen individual with habitual winners so that we can guarantee a loss for the randomly chosen individual. In essence, we need to identify individuals with low and high fighting abilities by actual, repeated performance, in the absence of external markers. Thus, we can identify habitual winners and habitual losers by staging repeated, serial contests and choose those individuals who never lose or never win, as our habitual winners and habitual losers respectively.

Whether we plan to use the method of self-selection or random-selection, it is very useful, indeed essential, to have a good knowledge about what traits determine fighting ability in the chosen study species. And these vary greatly between species and may include body size, age, being in or out of one's territory, and so on. Prior familiarity with the study animal, especially its natural history and general behaviour patterns, particularly its social behavior will be very helpful. Sometimes, researchers pay great attention to the experimental design, sample sizes, statistical analysis and modelling, but inadequate attention to the biology of the species, treating different species as interchangeable black boxes. Any attempts to answer specific questions or test specific hypotheses are best embedded in long-term studies of the natural history, ecology and ethology of the study species, even if the experiments are conducted in an 'artificial' laboratory setting. Long-term familiarity with the biology of the study species is often evident when researchers spontaneously refer to them as 'my species'!

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I will now describe four different studies that examined the possible presence of winner-and/or loser-effects and their ramifications.

## Pumpkinseed Fish *Lepomis gibbous* – Is There a Winner-effect and How Long Does it Last?

Ivan D. Chase and Constanza Bartolomeo of the departments of Sociology and Ecology and Evolution respectively, State University of New York at Stony Brook, and Lee Alan Dugatkin, then at the H Morgan School of Biological Sciences, Lexington, Kentucky, USA used the pumpkinseed sunfish, *Lepomis gibbous* (family: Centrarchidae, order: Perciformes) (*Figure* 1), a very common fish in north-eastern North America, to test for winner-effects (they did not explicitly test for loser-effects) [3]. They employed the method of random-selection by taking advantage of the fact that fish outside their territory are most likely to lose fights with territory owners. As they state in their paper, they simply collected some fish from a pond in their University campus, brought them to their laboratory and conducted the experiments. But before conducting the experiments, they had to solve a tricky problem—all fish look alike—and they needed a way to individ-

**Figure 1.** Pumpkinseed fish *Lepomis gibbous*, a very common fish in northeastern North America used by Chase, Bartolomeo, and Dugatkin (1994), to demonstrate the winner-effect and its decay time (Image Source: Shutterstock.com).

ually identify the different fish in their experiments—how else would they know if winners win again and again.

Individual identification of animals in behavioural experiments is a crucial part of the methodology. There is no universal way of achieving this. As we saw in several previous articles in this series, my students and I uniquely mark individuals of the Indian paper wasp *Ropalidia marginata* by applying spots of quick-drying enamel paints of different colours to different parts of their bodies. Researchers studying birds often put coloured rings on their legs.

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There is another problem that they had to solve before staging any contests between different fish. How does one decide who is the winner and who is the loser? When two pumpkinseed fish are put together in a tank, they fight, i.e., show aggressive behaviour. In this species, the aggressive acts comprise four different behaviours—displacement (chasing the opponent), nip (biting the opponent), butt (thrusting the closed mouth against the opponent's body) and a fourth kind that they call 'attacks-no-responses' (nip or butt but the opponent does not move or respond, unlike in the three previous acts of aggression). They declared a fish as the winner of a contest between two individuals if one of them showed 20 consecutive acts of any of the above mentioned four kinds of aggressive behaviours towards its opponent, without the opponent ever retaliating. Armed with individually identified fish and a criterion to declare winners and losers, the researchers conducted 41 trials involving four different fish in each experiment.

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For every trial, they used the following configuration of fish tanks. They used two fish tanks; let us call them Tank A and Tank B. Tank A was smaller and had two compartments, while tank B was bigger and had three compartments (*Figure* 2). For each experiment, they randomly chose four (never before used) fish and placed one of them in one of the compartments of the smaller tank and the other three, one each, in the three compartments of the larger tank. Let us call the four fish as 1, 2, 3 and 4 as in *Figure* 2. The other compartment of the smaller tank housed a different fish to be used for a different experiment, of no interest for us here (and hence is shown as a blank in *Figure* 2).

In order to use the method of random-selection, they needed a guaranteed loser so that the randomly chosen fish in the first contest will have a winning experience. They produced a guaranteed loser in the following way. They transferred fish 1 (from tank A) to the partition housing fish 2 in tank B. In this situation, fish 1

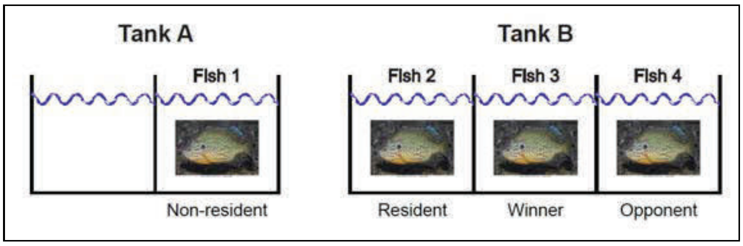


**Figure 2.** The experimental design used and the location of the four fish in the two tanks. See text for explanation (Reprinted with permission from D. Chase, C. Bartolomeo, and L. A. Dugatkin, Aggressive interactions and inter-contest interval: How long do winners keep winning? *Animal Behaviour*, Vol.48, pp.393–400, 1994.

is an intruder, outside its own territory while fish 2 is a resident, inside its own territory. As we noted above, it is very common that fish lose fights when they are in an alien territory and win fights when they are in their own territory, regardless of their intrinsic fighting abilities. So, as expected, fish 1 lost the fight, and fish 2 won the fight. At this point, they removed the winning fish 2 and let the losing fish 1 remain in that part of tank B. A fish that has just lost a fight is very likely to lose the next one too. This argument, you will realise assumes the existence of a loser-effect. Both winner-effects and loser-effects were already widely known (or expected) when this study was done, and so they could use that knowledge to design a suitable experiment to study winner-effects. Thus, fish 1 is now the almost guaranteed loser they needed.

At this point, they staged the first of two consecutive contests necessary to demonstrate the existence of a winner-effect. This they did by simply removing the partition between fish 1, which had remained in tank B, and its new neighbour, fish 3. As expected, fish 3 won the contest and fish 1 lost again. Their goal was to see if this winning experience that fish 3 got in this first contest would help it to win the next contest as well. In order to test this, they staged a second contest by re-introducing the partition between fish 1 and fish 3, removing the partition between the winning fish 3 and the naïve fish 4, and recorded the consequences.

Since they had used the method of 'random-selection', they could compare the numbers of wins and losses in the 2nd contest against a null hypothesis of 0.5. They did so by what is called a binomial test which tells you how much confidence you can have that the proportions of wins are greater than the proportions of losses. Notice that small imbalances in the proportions of wins and losses can, of course, occur by chance alone, especially when the sample sizes are small, as is often the case in these experiments. The binomial test computes the probability with which the observed deviations from 0.5 in the proportion of wins would be expected to occur by chance alone, i.e., in the absence of any winner effect.

*Results*

In the first 18 experiments, they staged the 2nd contest immediately after the 1st contest so that there was no delay that could bring about a decay in any winner effect, if it existed. The winner in the 1st contest again won the 2nd second contest in 14 out of these 18 trials (*Figure* 3). The binomial test indicates that the probability of getting this ratio of WW:WL to be 14:04, by chance alone, i.e., without any winner-effect, is 0.012 (or 1.2%) (traditionally represented as P = 0.012). This is quite a small probability, less than 0.05 which is the traditional cut-off point for rejecting the null hypothesis that there is no winner-effect and accepting the alternate hypothesis that there is a winner-effect. Hence they concluded that there is a winner effect in pumpkinseed fish *Lepomis gibbous*. The goal of their experiment was not only to demonstrate a winner effect but to see how long it will last or, how quickly might the winner-effect decay. Hence they conducted 12 new trials where they introduced a 15-minute delay between the first contest and the second contest. Now they obtained a WW:WL ratio of 10:02 which corresponds to P = 0.016 (*Figure* 3). Thus they concluded that the winner effect lasts for at least 15 minutes. Finally, they conducted another set of 11 trials with a delay of 60 minutes, always with fresh fish of course, and here they obtained a WW:WL ratio of 03:08, corresponding to P = 0.081—clearly, not significant (*Figure* 3). The winner effect had decayed within the hour.

Pumpkinseed fish *Lepomis gibbous* display a clear winner-effect which lasts less than an hour.

*Summary*

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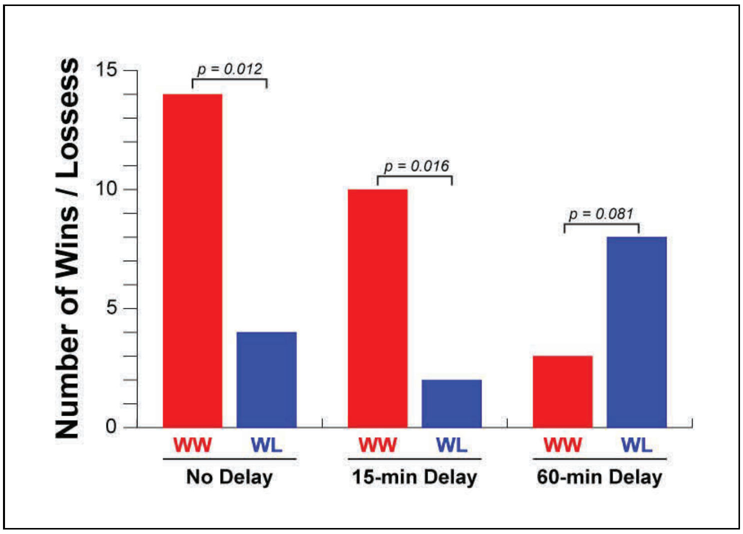


**Figure 3.** Results of the 2nd contest using winners of the 1st contest and pairing them with a randomly chosen naïve individual. The 2nd contest was staged immediately, after a delay of 15 minutes or a delay of 60 minutes. WW = winner of the 1st contest also won the 2nd contest; WL = winner of the 1st contest lost the 2nd contest. See text for details. (Figure drawn by the author using the numerical data from D. Chase, C. Bartolomeo, and L. A. Dugatkin, Aggressive interactions and inter-contest interval: How long do winners keep winning? *Animal Behaviour*, Vol.48, pp.393–400, 1994.)

## Mangrove Killifish *Rivulus marmoratus*—Are Multiple Experiences Integrated?

### *Background*

Yuying Hsu & Larry L. Wolf of the Department of Biology, Syracuse University, New York, USA, used the mangrove killifish *Rivulus marmoratus*, now renamed as *Kryptolebias marmoratus*, to study winner-loser effects. *Kryptolebias marmoratus* (family: Rivulidae, order: Cyprinodontiformes) is a common, small mangrove killifish that occurs in brackish or marine waters along the coast of Florida, Mexico, Central and South America (*Figure* 4). They can be amphibious and often hermaphroditic, and are often used as aquarium fish, growing to no more than about 4 cm. The name killifish sounds like they may be dangerous but they are not; their name comes from *killi* which means a ditch in Dutch! Hsu and Wolf conveniently obtained their experimental animals from laboratory stocks maintained in the department for many generations.

### *Experimental Design*

To test for winner- and loser-effects, they also used the method of random-selection but via a different route as compared to Chase

**Figure 4.** *Rivulus marmoratus*, now renamed as *Kryptolebias marmoratus*, a common, small mangrove killifish that occurs in brackish or marine waters along the coast of Florida, Mexico, Central and South America, used by Y. Hsu and L. L. Wolf (1999), to see if multiple experiences are integrated. (Image Source: Wikimedia Commons).

and colleagues (in the previous experiment with pumpkinseed fish), who you will recall, obtained the necessary guaranteed losers and winners by staging contests between pairs of fish, one inside its territory and the other outside its territory. In this study, the authors instead used the method of providing winning and losing experiences to the desired fish by pairing them with what they call ‘standard losers’ and ‘standard winners’. They obtained standard winners by staging a series of contests among several large fish and choosing the one that won all contests. Similarly, they obtained standard losers by staging a series of contests among several small fish and choosing the one that lost all contests. Thus, a randomly chosen fish was guaranteed to get a winning experience when paired with a standard loser, conversely, a randomly chosen fish was guaranteed to get a losing experience when paired with a standard winner. Now they could check whether such randomly chosen fish with winning or losing experiences would have higher than expected chances of winning and losing respectively, in their next contests, with other randomly chosen individuals.

Yuying Hsu and Larry Wolf, as the title of their paper [4] indicates, were interested not only in the effect of the most recent past experiences but in seeing how the fish might integrate the combined effects of multiple prior experiences, as they would likely have to do in nature. For starters, they decided to study the effects of two consecutive prior experiences on the final contest. They labelled the two consecutive prior experiences as 'penultimate experience' in the case of the first one and 'recent experience' for the second one, both before the final contest. By pairing randomly chosen individuals with standard winners and/or standard losers in two successive contest, they obtained individual with different experiences, such as WW, WL, LW, and LL, i.e., individuals that had experienced 2 successive wins, a win and a loss, a loss and a win and 2 successive losses. These were then engaged in a final contest with a different randomly chosen individual to study the effects of multiple prior experiences.

As in the previous study by Chase *et.al.*, [3], Hsu and Wolf [4] had to set criteria to declare winners and losers in a contest. This will necessarily depend on the species being used and experimenters should make a careful study of their model species under non-experimental conditions to decide the most appropriate criteria for their species. In this case, a fish was declared a winner if it chased and/or attacked its opponent for 20 minutes without retaliation. By this criterion, they obtained clear winners and losers in every contest, in one hour. As in the pumpkinseed fish experiment, they clipped the non-vascularized, outer margins of caudal fins and made sure there were no infections. Each experiment lasted 3 consecutive days during which the fish were given their 'penultimate' experience on day 1, their 'recent' experience on day 2 and the final contest was held on day 3. Recall that these authors used the method of random-selection and so they could compare their results against a null hypothesis of 0.5.

*Results*

They conducted 27 trials pairing WW individuals with LW individuals, i.e., individuals who had received a penultimate winning experience and also a recent winning experience with individuals who had received a losing penultimate experience and a winning recent experience. Notice that both individuals had a recent winning experience but differed in their penultimate experience. It turned out that WW individuals won significantly more contests as compared to LW individuals, suggesting that when both had the same recent experience, a winning penultimate experience

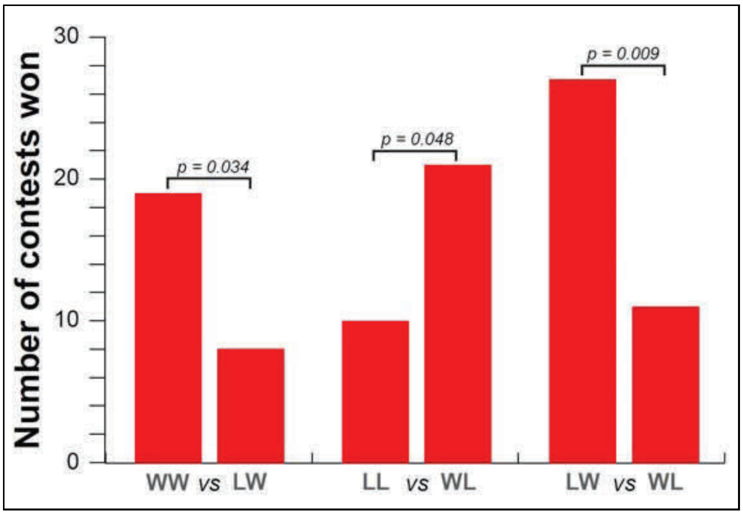


**Figure 5.** Numbers of contests won by different individuals in contests between individuals having different penultimate and recent winning and losing experiences. In the letters below that bars, W = winning experience and L = losing experience, while the first letter refers to the penultimate experience and the second letter refers to the recent experience. See text for details. (Figure drawn by the author using numerical data from Y. Hsu and L. L. Wolf. The winner and loser effect: integrating multiple experiences, *Animal Behaviour*, Vol.57, pp.903–910, 1999).

gave the fish an advantage over another who had a penultimate losing experience (see the first pair of bars in *Figure* 5).

Next, they conducted 31 trials with LL vs WL individuals. Notice that once again both contestants had a similar recent experience, albeit a losing one, and opposite penultimate experiences. This time, WL individuals won significantly more contests as compared to LL individuals showing once again that when the recent experience is the same, the penultimate experience gives an edge to an individual over another who had a penultimate losing experience (see the second pair of bars in *Figure* 5). Taken together, these results show that the winner and loser-effects not only last for two days but are not overturned by a more recent experience of the opposite kind.

When the penultimate and recent experiences are of the opposite kind, the recent experience trumps the penultimate experience.

Finally, they conducted 38 trials with LW vs WL individuals. Here the individuals differed from each other in both their penultimate and recent experiences and permit us to ask which is more important, the penultimate experience or the recent experience. As it turned out, and not so surprisingly, LW individuals had a significantly higher probability of winning the contest as compared to WL, clearly indicating that when the penultimate and recent experiences are of the opposite kind, the recent experience

**Figure 6.** Green Swordtail *Xiphophorus helleri*—Male aquarium fish, used by Beaugrand, Goulet and Payette (1991) to study if body size and winning/losing experience cancel each other and by Dugatkin and Druen (2004) to examine the social implications of winner-loser-effects. (ImageSource: Shutterstock.com)

trumps the penultimate experience (see the third pair of bars in *Figure* 5).

*Summary*

In the mangrove killifish *Rivulus marmoratus*, experience gained from at least two prior fighting experiences influence future fighting success. When two fish have identical penultimate winning or losing experiences but have differing recent experiences, the recent experience influences future fighting success. Conversely, when two fish have similar recent experiences but different penultimate experiences, their penultimate experience influences future fighting success.

## Green Swordtail Fish, *Xiphophorus helleri*

The next two studies, answering two different questions, have used the green swordtail fish *Xiphophorus helleri*. This is a brackish water, live-bearing fish, native to North and Central America. It has been introduced in many places and has become an invasive species and quite a nuisance, causing ecological damage, but it's also very popular aquarium fish (*Figure* 6).

## *Can Body Size and Experience Cancel Each Other?*

*Background*

Early studies of winner- and loser-effects often lamented that most studies of fighting and aggression focused only on the intrinsic fighting abilities and the resulting resource holding capacities of animals, and neglected to consider extrinsic factors such as the role of experience e.g., winner- loser-effects. Of course, both intrinsic and extrinsic factors are important. Once winner/loser effects were demonstrated in many species, people have begun to consider the possible interaction between intrinsic and extrinsic factors. In a set of simple experiments, Jacques Beaugrand, Claude Goulet and Daniel Payette, of the Department of Psychology at the University of Québec in Montréal, Canada, have simultaneously considered the effects of body size and prior winning or losing experience, on the success or failure during future contests [5]. Previous research on this species had already shown the effect of body size as well as prior experience, but these factors had only be considered separately. With the knowledge that both body size and prior experience are important, these researchers were able to design simple experiments to study the possible interaction of these factors. The fish they used, and indeed, many such common laboratory animals, are often available for purchase from animal breeders. As the author says, stocks of these fish were always available in their laboratory for various experiments, maintained in mixed groups of 100–150 adult and immature males and females in large tanks (90 × 50 × 40 cm).

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*Experimental Design*

When required for the experiments, adult male fish from these tanks were captured 'as randomly as possible' and the experimental tests staged in different glass tanks measuring 30 × 15 × 15 cm. Possibly because winner and loser effects had already been demonstrated in this species, they simply used the method of self-selection. Introducing two randomly chosen fish which were rather similar in body size into such tanks, they determined who was the winner and who was the loser, at the end of 12 hours. Let us label them as winners and losers, in keeping with the language we have been using in this article, although the authors themselves label them as dominant and subordinate. At the end of this, they separated the fish. On the following day, they staged a second contest using the following combinations of fish: (1) winners and losers of equal size, let us label them as EW and EL, (2) large winners versus small losers (LW vs SL), (3) small winners versus large losers (SW vs LL), (4) large winners versus small winners (LW vs SW) and (5) small losers versus large losers (SL vs LL). In these contests, they considered a fish as having won the contest if it successfully chased its opponent at least six times without being threatened, attacked or bitten by the opponent.

*Results*

Because these authors use the method of self-selection, they should have used the null hypothesis of 0.67 to check their results, as recommended and proved mathematically by the paper discussed above [2]. For reasons that are not entirely clear, the present authors nevertheless used 0.5 as their null hypothesis [5]. I will say more about this soon, but for the present, I will describe the results as described by the authors themselves using the null hypothesis of 0.5. In the 1st set (EW vs EL), when both fish had similar body size, the winner of the 1st contest clearly won the second contest too, and the loser of the 1st contest lost the 2nd contest too. We can, therefore, conclude that when body sizes are similar, prior experience of winning or losing in the first contest alone will determine the outcome of the second contest. Does this prove the existence of a winner-effect, a loser-effect, or both? This is an interesting question. The answer is that it either proves the existence of a winner-effect or the existence of a loser-effect but not necessarily both. If there is a winner-effect, a winner is expected to win again even against a randomly chosen individual. In other words, a winner will win again even if there is only a winner effect and no loser effect. Similarly, if there is a loser effect, a loser is expected to lose even against a randomly chosen individual. In other words, a loser will lose again even if there is only a loser-effect and no winner-effect. Thus, for this result to be

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obtained, there must be at least one of the two, a winner-effect or a loser-effect but not necessarily both. There may be both winner- and loser-effects, as we know from previous work, but it is important to realise that this particular experiment can only be taken to conclude the presence either of a winner-effect or of a loser-effect but not of both, and we cannot tell which one it is. In the second set when a large winner was pitted against a small loser (LW vs SL), the large winner won again. This could be a combination of the advantage of body size as well as the winner effect. In the third set, when a small winner was pitted against a large loser (SW vs LL), the large loser won showing that a large body size can overcome the ill effects of the loser effect. In the 4th set, when a large winner was pitted against a small winner (LW vs SW), the larger fish won, showing the pure effect of body size. In the 5th set, when a small loser was pitted against a large loser (SL vs LL), the large loser won, showing again, the pure effect of body size.

These cleverly designed sets of experiments demonstrate the existence of either a winner- or a loser-effect, the advantage of body size and that body size differences can override winner-loser effects. To be more precise, these experiments allow us to conclude that body size can counteract either the winner-effect or the loser-effect, but for similar reasons as discussed above, we cannot be sure that body size can overcome both winner-and loser-effects, but only that it can overcome at least one of them. I would like to encourage readers to think of how to design experiments (using self-selection), that will help us to demonstrate both winner-effects and loser-effects, rather than just one of them. Similarly, how to design experiments that check whether a body size can overcome both winner-effects and loser-effects, rather than just one of them.

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The results of all the five sets of experiments described above yielded statistically significant results, considering a null hypothesis of 0.5. But we have seen in the beginning of this article that a null hypothesis of 0.5 is not adequate to demonstrate winner- or loser-effects when the method of self-selection is being used. The relevant set here is the first set namely, EW vs EL, from which we concluded that there is either a winner-effect or a loser-effect. On the face of it, this conclusion is not valid because it used a null hypothesis of 0.5. I, therefore, recalculated the statistical significance of the first set using the null hypothesis of 0.67 and the difference is still significant! Luckily, we can still infer that there is either a winner-effect or a loser-effect. But this is just a matter of luck. We, therefore, have to be careful to use the correct null hypothesis. The conclusions of this experiment remain valid even with the null hypothesis of 0.67, for the 1st, 3rd and 4th sets. It falls below the required level of significance ($P < 0.05$) for the 2nd and 5th sets. Luckily, we are not inferring winner-or loser-effects from these sets but only the effect of body size for which a null hypothesis of 0.5 is adequate. Thanks to luck, all the three conclusions of these experiments namely, (i) there is a winner-or a loser-effect, (ii) there is an effect of body size and, (iii) body size can overcome either the winner-effect or the loser-effect, are valid.

When we say that body size can overcome winner- and/or loser-effects, how much difference in body sizes is required for this to happen? To answer this question, the authors of the study repeated the 3rd set from the previous experiment, pitting a small winner with a large loser (SW vs LL) several times with a range of differences in body size between winners and losers of the 1st contest. Body size may be tricky to measure in fishes, because as you might imagine, much depends on the shape of the body. In this case, it is known that the lateral surface area (LSA) is a good measure of body size especially in deciding the outcome of dyadic dominance interactions. For each fish in the experiment, they measured three parameters namely, (1) the total length (L, measured from the snout to the end of the caudal fin); (2) the flank height (H, measured from the base of the dorsal fin to the origin of the gonopodium), and (3) the sword length (S, measured from the end of the middle rays of the caudal fin to the tip of the sword). They measured these parameters with minimum disturbance to the fish, using a wire mesh partition in the tank with which they

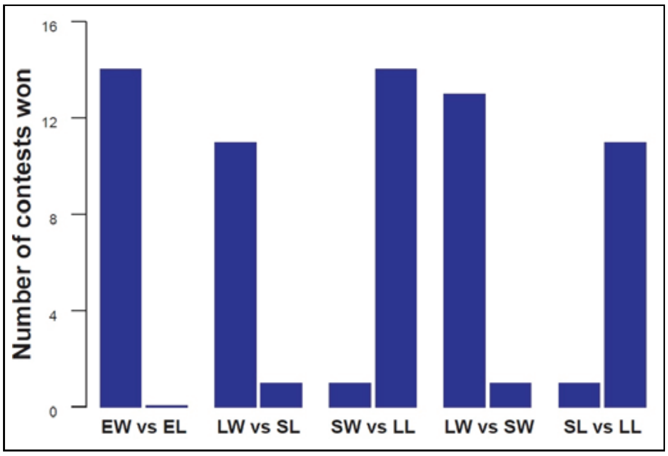


**Figure 7.** Effects of prior winning and losing experiences and of body size, in influencing the outcome of fights in the green swordtail fish, *Xiphophorus helleri*. In the X-axis labels, the first letters E (equal), L (large) and S (small) refer to the body size of the fish and the second letters W (winner) and L (loser) refers to winning and losing experiences respectively in the previous encounter that these fish participated in. The left-most pair, bars labelled as EW vs EL refers to a contest between equal sized winners and losers, the second pair of bars refer to contests between large winners and small losers, and so on. See text for details. (Figure drawn by the author using numerical data from J. Beaugrand, C. Goulet and D. Payette, Outcome of dyadic conflict in male green swordtail fish, *Xiphophorus helleri*: Effects of body size and prior dominance, *Animal Behaviour*, Vol.41, pp.417–424, 1991.)

could gently nudge the fish towards one side of the tank and mark its measurements on the glass wall of the tank with a felt-tip-pen. It is important to mention these little details so that others can use them or improve upon them. The lateral surface area was then computed as:

$$LSA = (L \times H) + S.$$

Next they computed the size difference between the members of a pair as:

$d$ = [(*LSA of the large fish - LSA of the small fish*)/*LSA of the small fish*] × 100.

This means they calculated the extent to which the large fish were larger than the small fish as a percentage of the body size of the small fish. In the experiments pitting small winners versus large losers, to see if a large body size can overcome the negative effects of the loser-effect or whether a small body size can negate the advantage of a winning effect, they paired the fish such that the large losers were from 0% to about 75% larger than the small winners. Is there a relation between the percentage size difference and the probability that the larger loser will defeat the smaller winner? Can we, therefore, predict the probability of success of the larger loser given the body size difference between it and the smaller winner? To answer these questions, they used a special kind of

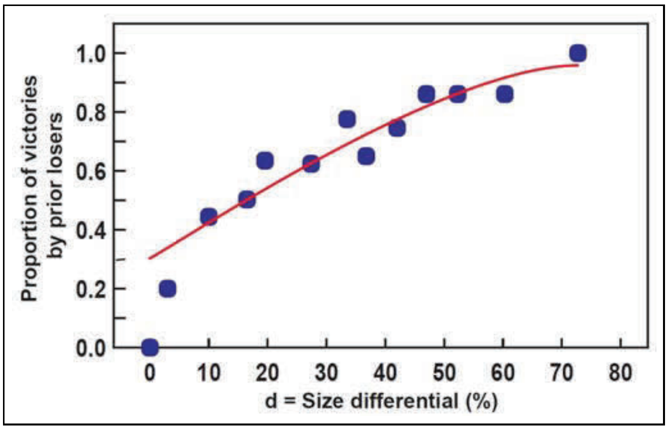


**Figure 8.** The relationship between the size differential and proportion of victories of the loser as given by the logistic function (red line). The points represent the proportion of conflicts won by the prior loser at successive 5% d-intervals. See text for details. (Reprinted with permission from J. Beaugrand, C. Goulet and D. Payette, Outcome of dyadic conflict in male green swordtail fish, *Xiphophorus helleri*: Effects of body size and prior dominance, *Animal Behaviour*, Vol.41, pp:417–424, 1991.)

regression analysis known as the 'logistic regression'. This is required when the dependent variable (probability of success, in our case) is not a continuous variable but a binary one, such that an individual may either win or lose with nothing in between. In this case, using the body size differential as the continuous independent variable, and the probability of victory for the loser as the binary dependent variable, they performed a logistic regression analysis which turned out to be highly significant ($P < 0.001$), meaning that the size differential is a good predictor of the outcome of these fights (*Figure* 8). We can see from the figure that when the size difference is small, there is not much chance that the previous losers will now win but as the size differential increases, there is a significant increase in the probability that the larger losers will win against the smaller winners. The effect of size differential on the probability of winning is gradual and linear and there does not appear to be any particular threshold difference required for the larger losers to defeat the smaller winners. As always, the 'winner' and 'loser' refers to winning and losing experiences respectively in the previous contest in which these individuals took part.

*Summary*

Based on this and previous studies we can conclude that in green swordtail fish, *Xiphophorus helleri*, fighting success is influenced both by body size as well as by previous winning and losing experiences and that these two effects can cancel each other out.

### *Do Winner- and Loser-effects Affect Hierarchy Formation?*

*Background*

As we have seen, all studies demonstrating winner- loser-effects conduct experiments with two individuals at a time. This is a bit artificial because animals in nature do not merely indulge in dyadic interactions. In many species, individuals organise themselves in dominance hierarchies and subsequent interactions depending on the position of respective individuals in the hierarchy. Using green swordtail fish, *Xiphophorus helleri* again, Dugatkin and Druen (2004) [6], therefore, investigated whether the winner- and loser-effects evident in dyadic interactions influence the position in the hierarchy that winners and losers occupy. If not, the winner-and loser-effects that we detect in dyadic interactions will have no relevance in the natural lives of these species.

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*Experimental Design*

Dugatkin and Druen used a simple experimental design. In the first part of the experiment, they prepared winners, losers and neutral fish, using the method of random-selection. As in previous experiments, a randomly chosen fish was made a winner by pairing it with a fish sufficiently smaller than itself. Conversely, a randomly chosen fish was made a loser by pairing it with a fish larger than itself. Individuals were labelled as winners if they made ten consecutive acts of aggression on their opponents without being attacked in turn by their opponents. Neutrals were randomly chosen fish that did not interact with any other fish. In the second part of the experiment, they brought together, in a single fish tank, a randomly chosen winner, a randomly chosen loser and a randomly chosen neutral, in order to observe the formation of a dominance hierarchy. The observed hierarchy was considered stable if the relative positions of the three individuals did not change over a period of three days. The question they were interested in concerned the relative positions of the winners, losers and neutrals in the hierarchy of three individuals.

*Results*

Repeating the experiment 20 times, they successfully observed stable hierarchy formation in 18 trials. In hierarchies of 3 fish of three types (winner, loser and neutral), we expect six types of hierarchies depending on the relative positions of the three kinds of fish in the three possible positions namely, top, middle and bottom of the hierarchy. These would be:

W-L-N, W-N-L, N-L-W, N-W-L, L-W-N, and L-N-W,

where, W = winner, L = loser and N = neutral and the positions from left to right would be top, middle and bottom of the hierarchy respectively. If there is no effect of the winner- and loser-effect on the positions occupied by the various fish in the hierarchy, then we would expect each of the six types of hierarchies at an equal frequency of 3/18. If on the other hand, being a winner, loser or neutral influenced a fish's position in the hierarchy, certain kinds of hierarchies should be seen more often than expected by chance alone, and others should be seen less often than expected by chance alone. Dugatkin and Druen found that the observed frequencies of the six different types of hierarchies differed significantly from 3/18, expected by chance alone. If both winner- and loser-effects have a strong influence on the positions of the fish in the dominance hierarchy then we would expect the winner in the dyadic interactions should be at the top of the hierarchy and the loser in the dyadic interactions should be at the bottom of the hierarchy while the neutral, i.e., the fish with no fighting experience should be in the middle of the hierarchy. Indeed, Dugatkin and Druen found that only the W-N-L type of hierarchies were significantly over-represented, with a frequency of 11/18 as compared to the chance expectation of 3/18. This was significant at $P < 0.001$ by a standard $\chi^2$ test with 5 degrees of freedom (*Figure* 9). Looked at in another way, winners were the top-ranked individuals in 13/18 of the hierarchies and losers were the bottom-ranked individuals, also in 13/18 hierarchies. These numbers were significantly greater than the chance expectation of 6/18 at $P < 0.05$ as judged by Fisher's exact test [6] (*Figure* 9). Notice that the chance expectation here

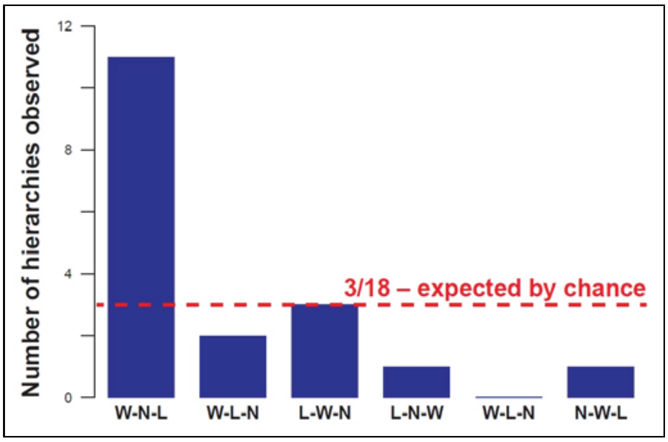


**Figure 9.** Frequencies of each of the six possible types of hierarchies observed (blue bars). The horizontal red line indicates the frequency for each type that would be expected by chance, i.e., if winner- loser-effects have no influence on hierarchy formation. Compared to this chance expectation, only the W-N-L hierarchies were over-represented. W = winner, L = loser and N = neutral, i.e., the fish had no prior experience of either winning or losing. W-N-L means the winner fish occupied the top position in the hierarchy, the neutral fish occupied the intermediate position in the hierarchy and the loser fish occupied the bottom position in the hierarchy, and so on. See text for details. (Figure drawn by the author using numerical data from L. A. Dugatkin, M. Druen, The social implications of winner and loser effects, *Proceedings of the Royal Society of London Series B (Suppl.)*, Vol.271, S488–S489, 2004.)

is 6/18 because, in the absence of any effect of previous experience, each type of fish (winner, loser or neutral) can be in each of the three positions (top, middle or bottom) in six of the 18 hierarchies. Clearly, the winner- and loser- effects had a significant influence in hierarchy formation showing that winner- and loser-effects that we detect in 'artificial' dyadic interactions have real-life consequences for the individuals involved. We can speculate that stable dominance hierarchies can be more easily established if there are strong winner- loser-effects so that individuals can sort themselves out without unnecessary, continued fighting.

*Summary*

Winner-and loser-effects do affect hierarchy formation.

## Reflections

All the experiments described in this article are not very hard to perform and do not require very much by way of facilities or expense, other than the ability to rear the animals in the laboratory or home. How many millions of people must be keeping fish in fish tanks as a hobby or for simple pleasure! No wonder fish have been one of the most favourite animal groups for studying winner- and loser-effects. It is also telling that so many studies, even by scientists, have erred in choosing the correct design of the experiments (self-selection vs random selection) and in inferring the correct predictions against which to test the results (0.5 vs 0.67, as the null hypothesis). We do need a book on 'how to design experiments', don't we?

In spite of the relative ease of conducting experiments to determine the presence or absence of winner- and loser-effects, and their ramifications and consequences for animals, this field is very much in its infancy. But the little that has been done shows that it is an extremely promising field. In a review of the literature conducted in 2006 Hsu *et.al.*, [7] discovered that winner-and/or loser-effects have been studied only in about 48 species of animals, including 6 insects, 3 arachnids, 3 crustaceans, 23 species of fish, one reptile, 5 birds and 7 mammals. I do not know how many such studies have been conducted since the publication of this review 14 years ago, but I am tempted to mention that one of my students Alok Bang, whom we met in the 8th article in this series, and I wondered why no one had looked for winner- and loser-effects in any social insect. We found this gap in the literature particularly surprising because social insects might be especially expected to benefit from having winner- and loser-effects because they live in colonies and need to interact with each other repeatedly. We remedied this situation by demonstrating that both winner- and loser-effects exist in the Indian paper wasp *Ropalidia marginata*. I must also add that this gives us a rather unique opportunity to study fighting by females while most of the other studies concern fighting by males only [8].

Imagine the opportunities available to study these interesting phenomena in many animal species from different branches of the evolutionary tree?

Imagine the opportunities available to study these interesting phenomena in many animal species from different branches of the evolutionary tree? When and why do winner-and loser-effects exist and when not and why not? Can only one of them, winner- or loser-effect exist without the other, and if so, what does that mean? How long do these effects persist and why? What intrinsic or other extrinsic factors can complement or counter-act winner and loser-effects? What is the consequence of winner- and loser-effects on the participation of individuals in their social life? Do winners and losers fight more and less, or do they sim-

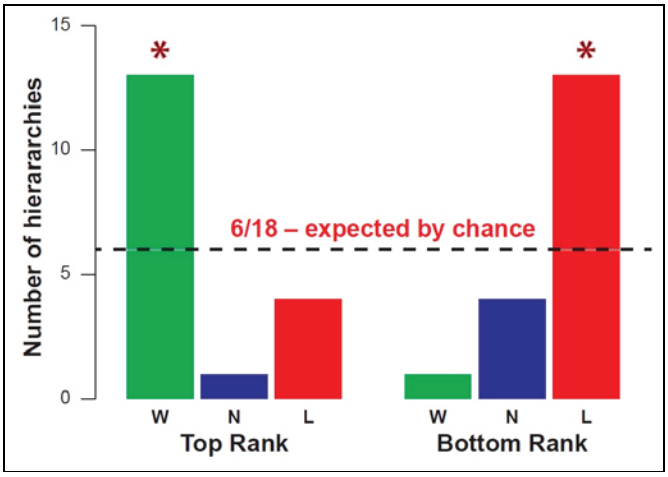


**Figure 10.** The observed frequencies of winner, neutral and loser fish at the top and bottom positions in the hierarchies (bars). The horizontal line represents the frequencies expected by chance alone, if winner- loser-effects had no influence on which position the fish occupied in the hierarchy. See text for details. (Figure drawn by the author using numerical data from L. A. Dugatkin, M. Druen, The social implications of winner and loser effects, *Proceedings of the Royal Society of London Series B (Suppl.)*, Vol.271, S488-–S489, 2004.)

ply win more and less? What effect does winning and losing have on their subsequent behaviour? How is the apparent increase or decrease in confidence manifested in their behaviour? How and when are multiple experiences integrated? How many prior experiences matter? We need a great deal of data to answer these questions in many different species to be able to take this field to the next step namely to understand the effects of these behavioural outcomes on the physiology of the animals. Even without studying physiology, there is a great opportunity to develop this into a field of comparative ethology if we study diverse taxa and take an evolutionary approach.

Consider the very large numbers of students and early career scientists who can exploit this relatively unpopulated field of research and conduct cutting-edge research at a trifling cost, using their favourite animal species. Here is a gold mine but in order to exploit it, we require a fundamental change in the behaviour of researchers. We need to stop jumping on the bandwagon of fashionable areas of research and have the courage to identify as yet unfashionable areas of research that have the potential to be made fashionable in the near future. This will require a different mindset, a different system of education, a different set of values, and a different system of evaluation, rewards and incentives. Are we up to the challenge?

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## Acknowledgements

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## Suggested Reading

**[1] R. Gadagkar, How to design experiments in animal behaviour – 10. Why do wasps fight? Part 2, *Resonance – journal of science education*, Vol.25, No.1, pp.111–131, 2020.**

**[2] J. Bégin, J. P. Beaugrand and R. Zayan Selecting dominants and subordinates at conflict outcome can confound the effects of prior dominance or subordination experience, *Behavioural Processes*, Vol.36, pp.219–226, 1996.**

**[3] I. D. Chase, C. Bartolomeo, and L. A. Dugatkin, Aggressive interactions and inter-contest interval: How long do winners keep winning? *Animal Behaviour*, Vol.48, pp.393–400, 1994.**

**[4] Y. Hsu and L. L. Wolf, The winner and loser effect: Integrating multiple experiences, *Animal Behaviour*, Vol.57, pp.903–910, 1999.**

**[5] J. Beaugrand, C. Goulet and D. Payette. Outcome of dyadic conflict in male green swordtail fish, *Xiphophorus helleri*: Effects of body size and prior dominance, *Animal Behaviour*, Vol.41, pp.417–424, 1991.**

**[6] L. A. Dugatkin, M. Druen, The social implications of winner and loser effects, *Proceedings of the Royal Society of London Series B (Suppl.)*, Vol.271, S488-–S489, 2004.**

**[7] Y. Hsu, R. L. Earley and L. L. Wolf, Modulation of aggressive behaviour by fighting experience: Mechanisms and contest outcomes, *Biol. Rev*., Vol.81, pp.33–74, 2006. DOI:10.1017/S146479310500686X**

**[8] A. Bang and R. Gadagkar, Winner-loser effects in a eusocial wasp, *Insect. Soc*., Vol.63, pp.349–352, 2016. DOI 10.1007/s00040-015-0455-x**

*Address for Correspondence*
Raghavendra Gadagkar
Centre for Ecological Sciences
Indian Institute of Science
Bangalore 560 012, India.
Email: ragh@iisc.ac.in