but rice will not make as effective use of the additional CO₂ as would other C₃ crops – those plants that, like 55% of grass species, have the basic mechanism of photosynthetic CO₂ assimilation. An alternative is to change rice into a C₄ plant, which could increase yield by up to 50% while using little or no more nitrogen or water. Although the challenges to such a venture are many and large, the reward for success would also be large. Rice genes will need to have their site(s) of expression changed, and additional genes from other plants introduced and expressed in the appropriate sites in a modified rice leaf anatomy. Research in this area is still at an early stage.

Can and should grass crops be produced for biofuels? As well as increasing problems with food security, we can look forward to a future with a scarcity of fuel, and especially the portable fuel currently used to power land, sea and air transport. Biofuels from grasses clearly can play a role in supplementing fossil fuels and energy from renewable sources, since ethanol from sugarcane has been used in fuels for motor vehicles in Brazil for almost 20 years, with the importance of bioethanol varying in parallel with the world price of oil. Least controversial as a means of increasing use of grasses in producing biofuels is the conversion of unused parts of grasses grown for human or animal food into biofuel, although not returning the unused part of grass crops to the soil will certainly deplete soil of organic matter. More contentious is the growth of highly productive grasses, usually perennial C4 grasses such as Miscanthus, specifically as fuel crops. Such uses will ultimately bring them into competition for land and resource inputs with food crops, and more generally with conservation areas and wilderness. All biofuel projects, and not just those involving grasses, need to be rigorously evaluated in terms of their lifetime carbon balance to avoid the possibility that unsuspected production of CO₂, for example in land use change, does not negate their role in CO₂ mitigation.

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Dogs showing separation-related behaviour exhibit a 'pessimistic' cognitive bias

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Up to five million pet dogs in the UK (~50% of the population) may, at some stage of their lives, perform undesirable separation-related behaviour (SRB) when left home alone [1], including vocalising, destruction and toileting [2]. Some owners perceive their dog to be 'fine' or even 'happy' when performing SRB [3], a few seek professional help [1], and others relinguish the dog [4]. Given the magnitude of the issue and the varied perceptions and behaviour of owners, the underlying emotional (affective) states of dogs showing SRB, and hence their welfare, requires elucidation. Whilst most dogs are believed to be anxious when showing SRB [1,2], it is uncertain whether their background affective state (mood) when they are not separated is also negative [1]. Here we use a new 'cognitive bias' measure of animal affect to show that dogs which exhibit high levels of SRB in a separation test also appear to have a more negative underlying mood.

The subjects were 24 dogs (50% male; estimated age range: 9-108 months) at two UK animal re-homing centres. Seven to twelve days after entering the centres, each dog took part in a previously validated SRB test [5] designed to measure its response to being left alone. Each dog was taken to a room where a researcher interacted with it for 20 minutes. The following day the dog was taken back to the room by the researcher who, after a short period of interaction, left it alone for five minutes during which the total time that the dog spent performing SRB was determined from video recordings and calculated as a 'SRB score' (for full details of the experimental procedures, see the Supplemental Information available online).

One to two days after the SRB test, each dog was subjected to a cognitive bias (CB) test of affective state, based on theoretical and empirical findings that an individual's background affective state, or mood, biases its decision-making and, specifically, that individuals in negative states make more negative ('pessimistic') judgements about ambiguous stimuli than happier individuals [6-8]. Dogs were trained to move from a start position to a food bowl on each trial of the CB test [9]. When the bowl was on one side of the room ('positive' location, P) it contained a small quantity of food, and when on the opposite side ('negative' location, N) it was empty (Figure 1A). When dogs were deemed to have discriminated P and N locations (see Supplemental Information), testing began. In test trials the bowl (empty) was placed at one of three ambiguous locations between P and N (near-positive (NP), middle (M), or near-negative (NN); Figure 1A). Three test trials were presented at each location with each test trial separated by four standard training (P, N) trials. Test trials allowed us to measure whether dogs ran quickly to the ambiguous locations (indicating anticipation of food: an 'optimistic' judgement) or more slowly (a 'pessimistic' judgement). We could thus investigate whether dogs showing higher levels of SRB also behaved more 'pessimistically', indicating an underlying negative affective state [6-9]. Mean latencies to get to the bowl during each of the three types of test trial (NP, M, NN), and during training trials (P, N) were calculated for each dog. To control for differences in dog size and running speed, we adjusted each dog's test trial latencies according to its mean 'baseline' latencies during training trials (for full details of the experimental procedures, see Supplemental Information).

Time spent performing separationrelated behaviour in the SRB test ranged from 0–169 seconds. SRB score was not affected by sex, neuter status, breed category, or animal centre and did not correlate with estimated age (see Supplemental Information). Figure 1B shows mean latencies to get to the bowl during training (P, N) and test (NP, M, NN) trials for all dogs. Bowl location affected latency (Friedman test, χ^2 = 68.15, n = 24, p < 0.001), reflecting a clear generalisation response with

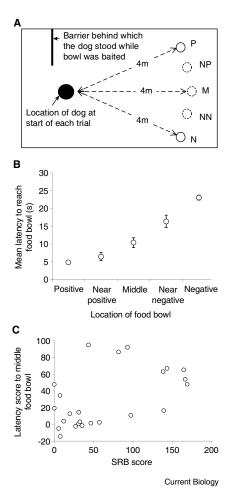


Figure 1. Schematic representation of the cognitive bias (CB) test, and graphs showing latencies to run to food bowls during the test.

(A) Plan view of the CB test set-up. One researcher held the dog on a lead behind the barrier at the start of each trial, while the other researcher, standing at the far end of the room, baited (or did not bait, depending on trial type) the food bowl. The bowl was then placed at one of the five locations (P, NP, M, NN, N), each of which was 4 m from the dog's start point (the two training locations are shown as circles with a solid line, and the three ambiguous test locations as circles with a broken line), and the dog was moved to the start point (black circle) facing towards the bowl locations. The dog was then released to approach the bowl. The side of the P and N locations was counterbalanced across dogs. (B) Mean latencies (+/- s.e.m.: unadjusted raw data) during the test phase on trials where the bowl was placed at the positive and negative training locations, and at the three test locations: near positive (NP), middle (M), near negative (NN). (C) Scattergram showing the relationship between the dogs' SRB scores and adjusted latency scores when presented with the bowl in the middle (M) test location.

dogs running fastest to the P location and progressively slower as the bowl location neared N. Dogs expressing more SRB behaviour showed a more 'pessimistic' judgement of ambiguous test locations. They had significantly higher adjusted latency scores when presented with the bowl in the M location ($r_s = 0.496$, n = 24, p = 0.014; Figure 1C), and a tendency towards higher latencies when the bowl was in the NN location ($r_s = 0.377$, n = 24, p = 0.069). Analysis of the raw (unadjusted) data produced similar results with significant positive correlations between SRB score and latency at both the M and NN locations (Spearman correlations, p < 0.05). SRB scores did not correlate with mean latencies to approach P or N during the test phase (Spearman correlations, p > 0.1), indicating that dogs with high SRB scores were not simply slower to run to all bowl locations. Further tests found no evidence that dogs with higher SRB scores were quicker to extinguish their responses to unrewarded test locations, and hence ran slower for this reason [6,10], that dogs' rates of extinction to unrewarded test locations explained the relationship between SRB and latency to test locations, that dog characteristics (sex, neuter status, breed category, estimated age) or location of the P bowl (right/left) affected latency to test locations, or that dogs relied on odour cues to detect whether the bowl was baited (see Supplemental Information).

Dogs at re-homing centres showing higher levels of SRB in a test that predicts subsequent SRB in the home situation also showed pessimistic-like behaviour in a cognitive bias test of affective state, which was unlikely to be explained by differences in running speed/motivation, learning ability, or other dog characteristics. Studies on a variety of non-human species indicate that, as in humans, pessimism is related to negative affect or mood ([7-9]; reviewed in [6]). Dogs showing SRB may thus also be in an underlying negative affective state, although the conscious experience of such a state cannot be known for sure [6,8]. This has implications for measuring and understanding the welfare consequences for dogs showing SRB, and may reveal

an underlying characteristic that predisposes individuals to perform anxiety-related behaviours, including in response to separation, which can be targeted in therapy to help minimise relinquishment, enhance welfare, and enhance the success of re-homing.

Supplemental Information

Supplemental Information can be found with this article online at doi: 10.1016/ j.cub.2010.08.030.

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