Comment

Supplementary information to:

How STRANGE are your study animals?

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Table S1 | Further examples for the seven categories of the STRANGE framework (for details, see notes beneath the table)

STRANGE categories	Suggested questions	Selected examples
Social background includes an animal's social status, the nature and frequency of its social interactions, and its past access to social-learning opportunities.	– *Does the test sample of subjects have an unusual social background?* – *What is the social rank of the subjects? Could this affect participation in experiments?* – *Are subjects housed alone or in groups? If in groups, of what size?* – *Are subjects tested alone or in the presence of other animals? Are testing conditions adapted for non-participating subjects?* – *What social experiences did subjects have prior to testing (e.g., of aggression, courtship, or mating)?* – *Could subjects have previously acquired information via social learning that affects their test performance?*	– Social rank affected innovation in chimpanzees[21]. – Dominance rank positively correlated with cognitive performance in starlings[22]. – Rearing density affected social information use and shoaling in guppies[23]. – Mating experience influenced courtship and mate competition in fruit flies[24]. – Social dominance interacted with social rearing condition to shape boldness and aggressiveness in skinks[25]. – Spatial discrimination ability positively correlated with social rank in male pheasants[26]. – Natural group size was positively correlated with cognitive performance in Australian magpies[27–29].
Trappability and self-selection are closely related processes that result, respectively, in individuals with certain characteristics (such as particular 'personality' types) being more likely to be trapped, or to participate voluntarily in experiments. Trappability effects are expected to be prominent in bio-logging studies where subjects are fitted with electronic tags for remote observation, while self-selection biases are a well-known – but usually neglected – problem in laboratory and field studies of animal cognition.	– *If animals are collected using traps, could this introduce sampling bias (e.g., by targeting bolder, more active, or hungrier individuals)?* – *Are different trapping methods used to avoid bias (e.g., different trap types, bait preparations, or trap placement strategies)?* – *If a self-selecting experimental design is used, do all potential subjects participate? What are the attributes of the non-participating subjects?* – *Can you rule out systematic bias in participation (e.g., by social rank, or personality type)?* – *Are test conditions adjusted to allow participation of otherwise excluded subjects (e.g., by amending the set-up or testing environment)?*	– Trappability of badgers varied between study sites, age and season[30]. – More exploratory and risk-taking flycatchers were more likely to enter traps[31]. – Bolder agamas entered traps sooner than shyer ones[32]. – Faster growing trout were more likely to be captured in nets[33]. – Faster exploring great tits were more likely to enter camera-equipped nest boxes[34]. – Pheasant chicks' self-selection in experiments varied with sex, condition, personality and experience[35]. – Self-selected participation in experiments was correlated with personality traits in squirrel

		monkeys[36]. – Sex, condition, and trap type affected trappability and trap-happiness of lampreys[37].
Rearing history describes an animal's developmental experiences, including the extent to which it has been exposed to a stimulating physical environment, other animals, and humans. Exposure to enrichment, social stimulation and exercise during development can affect brain development, and in turn, cognitive and motor performance.	– *Does the test sample of subjects have an unusual rearing history?* – *What is known about the origin of the subjects? Are they collected from the wild or captive-bred?* – *If captive-bred, were they raised by their parents (in species with parental care), by unrelated conspecifics, or by humans?* – *To what extent are subjects habituated to humans and testing environments?* – *To what extent have subjects experienced physical enrichment?* – *Are subjects housed alone or in groups?* – *If housed in groups, to what extent are these similar in size and composition to the groups these animals live in in nature?* – *Are differences in subjects' rearing history accounted for?*	– Female fruit flies reared alone were more aggressive[38]. – Male fruit flies from enriched environments had greater mating success[39]. – Enrichment enhanced spatial memory in mice[40]. – Hand-reared cranes were less vigilant than those reared by parents[41]. – Environmental variability promoted behavioural flexibility in cod[42]. – Environmental enrichment reduced habituation and problem-solving times in rattlesnakes[43]. – Enculturation affected tool-use performance in chimpanzees[44]. – Environmental enrichment was associated with 'optimistic' response biases in starlings[45]. – Environmentally-enriched salmon took fewer risks[46]. – Environmentally-enriched zebrafish were more aggressive[47].
Acclimation and habituation can result in behavioural changes over time, following handling, tagging, or exposure to novel testing situations.	– *Is the test sample of subjects unusual with regards to acclimation and habituation?* – *Could the behaviour of subjects be affected by the presence of a human observer?* – *Could the behaviour of subjects be affected by the presence of experimental equipment?* – *Do subjects have sufficient time to acclimate to captivity, and is acclimation time standardized?* – *Do all subjects acclimate to experimental conditions at the same rate?*	– Habituation to human observers reduced defensive behaviour over a period of days in Magellanic penguins[48]. – Ravens habituated to different modes of gaze following at different rates[49]. – Human observer presence reduced feeding and other behaviours in unhabituated baboons and macaques[50]. – Behaviour of damselfish took two days to stabilize after being brought into captivity[51]. – Reef fish gradually acclimated to the presence of

		cameras, but not to human observers[52].
Natural changes in responsiveness sometimes follow daily, reproductive or seasonal cycles, or the transition from one life stage to another. This means that the timing of experiments is often critical.	– *Is the test sample of subjects unusual with regards to any natural changes in responsiveness?* – *Is the study species known to exhibit diel, or seasonal variation in behaviour?* – *Is the timing of experiments standardized to account for possible effects due to time of day, photoperiod, or season?* – *Are all subjects of the same developmental stage, age and reproductive state? If not, how may this affect the behaviours observed?*	– White suckers had circadian activity patterns, and these were more stable in shoals[53]. – Young female guppies were more sensitive to model age when copying mate choice[54]. – Gravid female garter snakes were less active and sheltered in different landscape features[55]. – Cognitive performance and anxiety responses varied with estrous cycle in wild-type female mice[56]. – Reproductive state affected the strength of response to social information in sticklebacks[57]. – Zebra finches exhibited circadian rhythmicity of activity and singing[58]. – Enrichment affected memory in cricket nymphs, but not adults[59].
Genetic make-up can have profound effects on behaviour. There can be marked behavioural differences in genetic make-up between wild populations, and between wild and laboratory populations, often hampering attempts at broader generalization. Sex differences in behaviour are well documented.	– *Is the test sample of subjects unusual with regards to its genetic make-up?* – *Is the test sample of subjects sex-biased?* – *Are the subjects from a specific genetic line?* – *Is the line chosen suitable for examining the behaviour of interest? In other words, can artificial selection (or lack of natural selection) have affected behavioural competency or test performance?* – *If the subjects are wild-type, is the source population known?* – *Are inferences explicitly linked to the genetic line or study population investigated?*	– Fear responses differed between divergent strains of Japanese quail[60]. – Genetic strains of mice differed in exploratory behaviour and cognitive performance[61]. – Population-level variation in male fruit fly courtship songs had a genetic basis[62]. – Males and females differed in their responses to predator cues in a strain of mice[63]. – Differences in boldness and anti-predator behaviour between wild and domestic zebrafish had a genetic basis[64]. – Natural selection via predation drove differences in shoaling behaviour between guppy populations[65]. – Different strains of the parasite *Toxoplasma gondii* had differing effects on host behaviour[66].

		– Behavioural syndromes varied between natural populations of the delicate skink[67].
Experience encompasses opportunities for individual learning, such as participation in earlier experiments. Long-lived animals can accumulate complex experimental histories in research laboratories, which must be documented and accounted for.	– *Does the test sample of subjects have unusual experience?* – *Have subjects participated in similar earlier experiments that may affect test performance?* – *Have subjects participated in different experiments that may affect test performance?* – *Have subjects experienced husbandry procedures that may affect their behaviour?* – *Have subjects accrued experiences in the wild that may affect test performance?* – *Have subjects had previous opportunities to learn that may overshadow learning in the present study?* – *Are differences in subjects' experience accounted for?*	– Experience of clustered versus dispersed food shaped social-foraging behaviour of pollock[68]. – Experience of predator cues affected anti-predator behaviour of freshwater snails[69]. – Repeated disturbance of laboratory-housed Poeciliid fish increased their boldness[70]. – Tadpoles expressed time-of-day specific anti-predator behaviour based on experience[71]. – Learning one solution to an experimental task inhibited learning of alternative solutions in chimpanzees[72]. – Magpies recognized individual humans and behaved differently towards them[73]. – Defensive behaviour of gopher tortoises varied with experience of human disturbance[74]. – Exposure to novel prey reduced wariness towards different novel prey in great and coal tits[75]. – Experience of courtship and mating altered personality traits in sticklebacks[76].

The STRANGE framework collates a suite of factors that can affect animal behaviour; the acronym stands for: Social background, Trappability and self-selection, Rearing history, Acclimation and habituation, Natural changes in responsiveness, Genetic make-up, and Experience. As noted in the main text, these factors are often the focus of well-designed research projects, like the ones listed in this table, or are confounds that have been explicitly controlled for. But problems arise whenever samples of study subjects are biased with regards to one or several of the seven categories, and researchers do not account for this. Such unexplained variation can significantly impact the interpretation of experimental outcomes, limit the generalizability of findings, complicate comparisons between studies, and hamper reproducibility. We therefore recommend that researchers routinely ask themselves: *Are my animal subjects unusual – or strange – when compared to the wider population for which I wish to make inferences, in any of the seven categories of the STRANGE framework?* In this table, we suggest a non-exhaustive set of additional, category-specific questions researchers may find useful when trying to mitigate, or detect, STRANGE-related biases when designing their experiments or interpreting their findings (for a step-by-step guide to using the STRANGE framework, see Box S1). Note that there is overlap and strong interdependence between some STRANGE categories, and that for some of the examples listed here, several may apply. Examples were chosen to cover a broad range of taxa and study contexts, and are listed chronologically by publication date.

Box S1 | The 3D approach to using the STRANGE framework – recommendations for researchers and journals

(1) DESIGN

Consult the ARRIVE guidelines[16] and STRANGE framework (this article) when planning studies.

(2) DECLARE

Include the following text in journal author guidelines or reporting summaries:

(a) Provide detailed information – as applicable – on the origin (incl. trapping method), sex, age/developmental stage, mass/body condition, social status, personality type, housing conditions (incl. social contacts and enrichment), past opportunities for individual and social learning, experimental history, and testing protocols (incl. social context), for:

- *the final sample of subjects contributing data to the study; and*
- *the subjects that were part of the original sample, but did not contribute data (describe reasons for exclusion).*

(b) Evaluate scope for sampling biases based on the declarations made under (a), especially with regards to subjects' origin, self-selection behaviour, and prior experience.

(c) Describe what efforts (if any) were undertaken to mitigate potential sampling biases, especially with regards to sourcing representative subjects (such as using a variety of trapping methods), or adjusting experimental protocols to suit non- or slowly-engaging individuals.

(3) DISCUSS

Summarize the declarations in step (2) in two brief statements in the main text of research articles: one in the Methods section evaluating the STRANGEness of the test sample, and another in the Discussion section explaining how potential biases may limit the generalizability of the reported findings.

Supplementary References

21. Reader, S. M. & Laland, K. N. *Int. J. Primatol.* **22**, 787–805 (2001).
22. Boogert, N. J., Reader, S. M. & Laland, K. N. *Anim. Behav.* **72**, 1229–1239 (2006).
23. Chapman, B. B., Ward, A. J. W. & Krause, J. *Anim. Behav.* **76**, 923–929 (2008).
24. Saleem, S., Ruggles, P. H., Abbott, W. K. & Carney, G. E. *PLoS ONE* **9**, e96639 (2014).
25. Riley, J. L., Noble, D. W., Byrne, R. W. & Whiting, M. J. *R. Soc. Open Sci.* **4**, 161082 (2017).
26. Langley, E. J., van Horik, J. O., Whiteside, M. A. & Madden, J. R. *R. Soc. Open Sci.* **5**, 171475 (2018).
27. Ashton, B. J., Ridley, A. R., Edwards, E. K. & Thornton, A. *Nature* **554**, 364–367 (2018).
28. Ashton, B. J., Ridley, A. R. & Thornton, A. *Learn. Behav.* **47**, 277–279 (2019).
29. Smulders, T. V. *Learn. Behav.* **47**, 275–276 (2019).
30. Tuyttens, F. A. M. *et al. J. Appl. Ecol.* **36**, 1051–1062 (2019).
31. Garamszegi, L. Z., Eens, M. & Török, J. *Anim. Behav.* **77**, 803–812 (2009).
32. Carter, A. J., Heinsohn, R., Goldizen, A. W. & Biro, P. A. *Anim. Behav.* **83**, 1051–1058 (2012).
33. Biro, P. A. *Oecologia* **171**, 339–345 (2013).
34. Stuber, E. F. *et al. Behav. Ecol.* **24**, 1092–1098 (2013).
35. van Horik, J. O., Langley, E. J., Whiteside, M. A & Madden, J. R. *Behav. Processes* **134**, 22–30 (2017).
36. Polgár, Z., Wood, L. & Haskell, M. J. *Am. J. Primatol.* **79**, e22639 (2017).
37. Reinhardt, U. G & Hrodey, P. J. *Fishes* **4**, 34 (2019).
38. Ueda, A. & Kidokoro, Y. *Physiol. Entomol.* **27**, 21–28 (2002).
39. Dukas, R. & Mooers, A. Ø. *Anim. Behav.* **66**, 741–749 (2003).
40. Frick, K. M. & Fernandez, S. M. *Neurobiol. Aging* **24**, 615–626 (2003).
41. Kreger, M. D., Estevez, I., Hatfield, J. S. & Gee, G. F. *Appl. Anim. Behav. Sci.* **89**, 243–261 (2004).
42. Braithwaite, V. A. & Salvanes, A. G. *Proc. R. Soc. B* **272**, 1107–1113 (2005).
43. Almli, L. M. & Burghardt, G. M. *J. Appl. Anim. Welf. Sci.* **9**, 85–109 (2006).
44. Furlong, E. E., Boose, K. J. & Boysen, S. T. *Anim. Cogn.* **11**, 83–97 (2008).
45. Matheson, S. M., Asher, L. & Bateson, M. *Appl. Anim. Behav. Science* **109**, 374–383 (2008).
46. Roberts, L. J., Taylor, J. & de Leaniz, C. G. *Biol. Conserv.* **144**, 1972–1979 (2011).
47. Woodward, M. A., Winder, L. A. & Watt, P. J. *Fishes* **4**, 22 (2019).
48. Walker, B. G., Dee Boersma, P. & Wingfield, J. C. *Conserv. Biol.* **20**, 146–154 (2006).
49. Schloegl, C., Kotrschal, K. & Bugnyar, T. *Anim. Behav.* **74**, 769–778 (2007).
50. Iredale, S. K., Nevill, C. H. & Lutz, C. K. *Appl. Anim. Behav. Sci.* **122**, 53–57 (2010).
51. Biro, P. A. *Anim. Behav.* **83**, 1295–1300 (2012).
52. Nanninga, G. B., Côté, I. M., Beldade, R. & Mills, S. C. *Ethology* **123**, 705–711 (2017).
53. Kavaliers, M. *Can. J. Zool.* **58**, 1399–1403 (1980).
54. Dugatkin, L. A. & Godin, J. G. J. *Behav. Ecol.* **4**, 289–292 (1993).
55. Charland, M. B. & Gregory, P. T. *J. Zool.* **236**, 543–561 (1995).
56. Walf, A. A., Koonce, C., Manley, K. & Frye, C. A. *Behav. Brain Res.* **196**, 254–260 (2009).
57. Webster, M. M. & Laland, K. N. *Proc. R. Soc. B* **278**, 619–627 (2011).
58. Wang, G., Harpole, C. E., Trivedi, A. K. & Cassone, V. M. *J. Biol. Rhythms* **27**, 145–155 (2012).
59. Mallory, H. S., Howard, A. F. & Weiss, M. R. *PloS ONE* **11**, e0152245 (2016).
60. Mignon-Grasteau, S. *et al. Behav. Processes* **61**, 69–75 (2003).
61. Brooks, S. P., Pask, T., Jones, L. & Dunnett, S. B. *Genes Brain Behav.* **4**, 307–317 (2005).
62. Etges, W. J., Over, K. F., De Oliveira, C. C. & Ritchie, M. G. *Anim. Behav.* **71**, 1205–1214 (2006).
63. Adamec, R., Head, D., Blundell, J., Burton, P. & Berton, O. *Physiol. Behav.* **88**, 12–29 (2006).
64. Wright, D., Nakamichi, R., Krause, J. & Butlin, R. K. *Behav. Genet.* **36**, 271–283 (2006).
65. Huizinga, M., Ghalambor, C. K. & Reznick, D. N. *J. Evol. Biol.* **22**, 1860–1866 (2009).
66. Kannan, G. *et al. Folia Parasitol.* **57**, 151–155 (2010).
67. Michelangeli, M., Chapple, D. G., Goulet, C. T., Bertram, M. G. & Wong, B. B. *Behav. Ecol.* **30**, 393–401 (2019).
68. Ryer, C. H. & Olla, B. L. *Anim. Behav.* **49**, 411–418 (1995).
69. Turner, A. M., Turner, S. E. & Lappi, H. M. *Anim. Behav.* **72**, 1443–1450 (2006).
70. Brown, C., Burgess, F. & Braithwaite, V. A. *Behav. Ecol. Sociobiol.* **62**, 237–243 (2017).
71. Ferrari, M. C. & Chivers, D. P. *Anim. Behav.* **78**, 11–16 (2009).
72. Hrubesch, C., Preuschoft, S. & van Schaik, C. *Anim. Cogn.* **12**, 209–216 (2009).
73. Lee, W. Y., Choe, J. C. & Jablonski, P. G. *Anim. Cogn.* **14**, 817–825 (2011).
74. Bateman, P. W., Fleming, P. A., Jones, B. C. & Rothermel, B. B. *Behaviou*r **151**, 1267–1280 (2014).
75. Adamová-Ježová, D., Hospodková, E., Fuchsová, L., Štys, P. & Exnerová, A. *Behav. Processes* **131**, 24–31 (2016).
76. Monestier, C. & Bell, A. M. *Proc. R. Soc. B* **287**, 20192936 (2020).