Timothée Bonnet Comments	Responses
L.99 In addition to phenotypic plasticity and evolutionary change I like to consider changes in the demographic structure for instance changes in the distribution of age, sex or stage classes in the population. Demographic structure is sometimes seen as a component of phenotypic plasticity, or more often seen as a simple confounder to be corrected for in quantitative genetic studies. Changes in the demographic structure seem quite likely when a population is taken from the wild to live ex-situ, so it has the potential to explain phenotypic changes (which you may or may not expect would be reversed if the population is reintroduced into the wild). Therefore I think it would be good to consider demographic structure explicitly (not just as a confounder) in the context of the article.	Thank you, we have added demographic change as a potential driver of differences.
L.122-124 You may add other possible important consequences: altered ecological role of the organism, or mismatch between the properties of the organism and societal expectations.	Thank you, we agree this is a good point that will strengthen our argument here. Sentence below is now included. **Line 124-125:** Further, change in captivity could alter the ecological role of the organism with cascading consequences or the societal value of organism.
L.139 After a paragraph on molecular genetic variation, I was confused by the end "through monitoring of both genetic and phenotypic variation". You should be more specific / conceptually consistent. On the one hand there is a contrast between (quantitative) genetic vs. phenotypic variation (phenotypic variation being caused by genetic and non-genetic variation). On the other hand there is a contrast between molecular genetic variation vs. quantitative genetic variation, with quantitative genetic variation being phenotypic variation ascribed to (molecular) genetic variation.	Thank you, we have changed this last sentence to make it more specific and closely aligned to what we mean here. **Line 136-138:** Thus, ideally, *ex situ* populations are managed through monitoring of overall molecular genetic variation, quantitative genetic variation (the phenotypic variation ascribed to molecular genetic variation), and the non-genetic causes of phenotypic variation.
L.145 To use quantitative genetic methods you don't necessarily need a pedigree. Instead, you need a way to estimate pairwise additive relatedness. That can be done using a pedigree, partial information on kinship, or molecular markers (e.g., GRM computed from SNPs).	Thank you, this is a good point and will broaden the datasets that the techniques we outline can be used on.
L.183 Maybe clarify to "[reduced] genetic variation and [increased] inbreeding" ?	Thank you, done.

L.191-196 Managers could also use estimated breeding values to drive change in breeding values in the desired direction through selective breeding.	Thank you, we agree this is possible. We have added it as a possibility, but with caution. Perhaps, we are over worried that selective breeding could have unintended consequences (correlated trait changes, reduction of genetic diversity, selection for traits that attract human attention but aren't beneficial for wild reintroduction)
L.199-200 Statistical power will also depend on the structure of the pedigree and its covariation with confounding variables (e.g., maternal effects).	Thank you, we have noted this now. **Line 219-220:** …, the structure of the pedigree, and covariation of relatives with confounding variables (e.g. maternal effects, rearing facility).
L.204-206 Maybe I am missing the point, but isn't it trivial to document a change in average phenotype? If you cannot infer the causes of the change I am not sure it is really useful (for reasons you highlighted above: the phenotypic trend may be the result of different genetic changes and environmental changes.)	Thank you, we think you are right here. Upon reflection the last part of this paragraph is not really necessary. Stating that we cannot infer much without statistical power is perhaps apparent.
L.234 Isn't the section more about "Changes in plasticity" rather than "changes caused by plasticity" ?	Thank you, we have changed this heading to "Plasticity and changes in plasticity".
L.251-252 I am not sure that it true. If you could show that an environmental variable *causes* a change in the mean value of a trait expressed only once in life, wouldn't you say that you have infered a plastic response? That being said, I agree that it is often easier to study the plasticity of traits expressed multiple time in life.	Thank you, we think you are correct too. We have adjusted the wording so that it now says most easily measured. We have also discussed IxE and GxE distinctly. **Line 269-272:** For non-clonal species, we can most easily measure the plastic responses of labile traits that are expressed multiple times in an individual's life (annual fecundity, timing of breeding, migratory urge).
L.263-271 I am no expert, but I think there are some good examples of anti-predation behaviours that can be lost in captivity and hard to re-learn in introduced population. Maybe a good example to give here.	Thank you, we included this as an example and reference some of the literature investigating training and loss of anti-predation behaviours. **Line 289-292:** Finally, anti-predator behaviours will be valuable to monitor as they are sometimes, but not always, observed to disappear over time in captivity (Cox & Lima 2006; Blumstein et al. 2002) and anti-predator behavioural training may help improve survival upon release (Reading et al. 2013; Griffin et al. 2001; but see Moseby et al. 2012)
L.289-293 Maybe clarify what is the output of the SQuID and why it is relevant in this context?	Thanks, we no longer mention SQuID here and just mention general power analyses here now because there is not much point introducing the name of

| | this group. The name isn't relevant and just introduces an unnecessary acronym.

Line 304-311: Power analysis could be used to design data collection protocols that will ensure results can help improve a management programs ability to detect plasticity or whether an existing data set is adequate to statistically detect plasticity (Allegue et al. 2017). |
|---|---|
| Box 1 L.4 I am not sure the intercept indicates the average trait value. Isn't it the case only if the environmental variable is mean-centered? | Thank you, yes. We have clarified this now.

Box 1 – Line 3: When the environmental variable in such an analysis is mean-centred the intercept of such a line indicates the average trait value of an individual and the slope connecting the environment-specific trait values indicates the individual's response to captivity (Fig. 2). |
| L.351-353 3 spaces are missing: "value.Studboooks", "events,generate" and "tables.to". | Thank you, fixed. |
| L.405 I may have written the interpretation of additive genetic variance in fitness wrong somewhere, but a more accurate statement would be "the rate of genetic evolution [due to selection]" or "the rate of [adaptive] genetic evolution". | Thank you, the sentence now reads as below

Line 416-417: "The additive genetic variance of fitness should be equivalent, in theory, to the rate of adaptive genetic evolution..." |
| L.407 again, "how quickly [adaptive] genetic evolution". | Thank you, we added the word adaptive. |
| L.696 Don't you want some caption for figure 2? | Thank you, a caption has been added and the figure has been split into two. |
| Figure 3: I don't understand how (D) is supposed to illustrate a subcase of (C). To me it looks like all families in (D) have the same plastic response and differ only in elevation, whereas (C) illustrated differences in plastic responses. | Thank you, I think this situation can arise if all of the differences among individuals (in C) are permanent environmental effects and not caused by additive genetic differences. We have added a little more text to clarify this.

Line 752-759: Variation in plastic responses to captivity. If there is a plastic response at the population level (A) individuals might all have the same plastic response (B) or they could differ in their responses to captivity (C). If individuals differ in their responses, these differences could be caused completely by environmental differences and we would not see differences among family groups (D) or genetic differences might also have differences among families contributing to observed differences among individuals (E). We illustrate differences in responses as if they were |

	completely caused by environmental (D) or genetic differences (E), but they can be caused by a combination of both environmental and genetic differences.
L.709 Shouldn't it be "Figure 5" instead of "Fig 4" ?	Yes, thank you, this has been fixed now.

Jesus Fernandez Martin Comments	**Responses**
Simplifying a bit, or being pragmatic, the required change of paradigm in the management of (exitu) conservation populations is to go beyond the classical idea of maintaining general (neutral) genetic diversity and including adaptive variation into the goals and criteria. Obviously, if we maintain plenty of general genetic variation, hopefully also adaptive variation will remain (drift affects the whole genome). However, if there is information on genetic conformation for fitness-related traits the probability of survival of the population will be higher by including it into the management procedures. The new philosophy would be performing some kind of 'selection' for key traits while maintaining general genetic diversity. This is the objective of the Optimal Contribution (OC) theory (Meuwissen 1997, J. Anim. Sci. and posterior developments) that it is common in breeding programs of commercial populations. This OC could be mentioned in the paper to widen the methodological options for managers. Actually, fitness is already accounted for in the management of conservation programs when (mono)genetic defects appear by avoiding the use of carriers of the deleterious allele. The new situation implies dealing with more or less infinitesimal traits and, thus, the need of applying quantitative genetics methods.	Thank you, we have tried to address this overall comment throughout and with the requested changes below.
A large amount of the submitted text is devoted to the relevant problem of plasticity. However, I think that in the manuscript plasticity is mixed and somehow confounded with GxE interaction. The latter is related with adaptation to captivity (even if both environments are stable) as the relative performance between genotypes may be different in captivity and the wild and, in the extreme, the best fitted genotype exsitu has a poor performance	

in the wild. GxE interaction can be dealt with more easily by performing multitrait evaluations (phenotype in captivity and nature considered different traits). Then captive individuals can be evaluated for their expected performance in the wild and used as parents of the next generation (or not). No need to measure the same individual in captivity and the wild but having relatives in both environments. For example, a parent can be evaluated by their released offspring.	
Contrarily, detecting the effects of captivity on the plasticity of individuals would imply a greater sampling/recording effort. The feasibility of the use of plasticity as a criterion should be critically included in the manuscript. For example: how to determine the key traits for adaptation/plasticity in the wild? What are the required sampling sizes to obtain enough accuracies? In the end, everything is related to the importance of planning the captivity environment to minimise the differences with the wild (and to what extent this is possible).	
Anyway, in the absence of further information, aiming at neutral variation minimises drift, the changes in allelic frequencies and the adaptation to captivity. Thus, it is not as bad as implicit in some of the comments in the text.	
The study would benefit from the inclusion of a (brief) discussion on the extra advantages that molecular information could provide to the One Plan Approach management. For example (but not limited): i) determining founder relationships in exsitu populations; ii) trace lineages from released individuals (descendants of reintroduced); iii) detect genomic regions involved in adaptation (differences between wild and captive populations).	Thank you, yes this is a very good point we have added a note near the beginning of the review that genomic data will help broaden the species quantitative genetic analyses could be used on and improve information in existing breeding programs.

Line 192-199: Our review is timely because recent genomic tools will make quantitative genetic analyses possible in a broader range of species and populations (Gienapp et al. 2017; e.g. Gervais et al. 2019). Genomic relatedness matrices can now be used in lieu of a pedigree relatedness and implemented in an animal model approach to estimate the additive genetic variances of traits in species where it would not have been possible to gather observational pedigrees or set up experiment breeding designs. Further, genomic tools can help to clarifying relationships among founding individuals in a population and connect descendants of released individuals to lineages in the captive population. |

I would also want to point out to a problem on the format of the manuscript. Throughout the manuscript plenty of redundancies can be found, with the same idea presented several times. This enlarge (unnecessary) the text and makes the reading more tiring.	
- Lines 44-45: I would include in the list those captive populations created in specific centres (i.e. for single species) not fitting the traditional definition of zoos. I think that such populations offer more opportunities for the management and more powerful actions can be conducted.	Thank you, we've included reference to centres that are specialized on the breeding or recovery of single/specific species. **Line 38-42:** Traditionally, species conservation planning has followed parallel but separate tracks: field biologists and wildlife managers' efforts to address conservation needs *in situ*, zoo, aquarium, and species-specific breeding centres (e.g. the United States Fish and Wildlife Service Black-footed Ferret Conservation Center), efforts to develop sustainable *ex situ* populations.
- Line 51: I think you should be more precise with the expression 'the potential for reduced reproductive fitness' when listing the challenges in the management of ex situ populations by highlighting that you mean the failure to reproduce in the wild once released (adaptation to captivity). Because, in general, ex situ populations perform reproductively well, mainly due to the benign environment. Moreover, as the next part of the sentence says 'adaptation to captivity' it may lead to think that they are different factors. If you mean loss of fitness due to inbreeding depression, I think that the problem of inbreeding is usually worse in the wild than in (well) managed ex situ populations.	Thank you, this is very helpful and will clarify the argument we are trying to make. We really meant to highlight that recovery efforts could be made more difficult by evolutionary forces occurring during the captivity management. We tried to make this more specific by noting that genetic drift, inbreeding, and adaptation in captivity could result in individuals less adapted for wild conditions. **Line 49-51:** The reproductive fitness of individuals released to the wild could be reduced by genetic drift, inbreeding, and adaptation that might occur in captivity (Frankham 2008).
- Line 65: This citation is missing in the References section.	Thank you, I have now added the Princée reference.
- Line 70: The concept of 'realities' could be a bit expanded commenting on the conflict optimal vs. feasible as well as the problem of the expected vs. the observed outcomes. The latter sometimes arises because of some factors not accounted leading to evolutionary changes (line 72) due to unintentional selection (line 74).	Thank you, we have expanded on this a little bit. **Line 70-74:** Optimal breeding designs will not always be feasible given a breeding program's resources and outcomes of any given captive management plan could deviate from expectations because of unaccounted for factors. Deviation from an optimal design either because it is not feasible or because of unaccounted for factors could lead to evolutionary change.
- Lines 75-76: This is what I expressed in my general comments. Not only neutral unspecific	

diversity should be preserved by minimising coancestry, but also data on adaptive diversity should be included in the management criteria.	
- Line 94: There is no Section 6. There is a mismatch between number of sections and the description of the contents.	Thank you, this has now been corrected.
- Line 156: The average breeding value is a parameter of a trait, not of a phenotype.	Thank you, phenotype has been changed to trait.
- Line 164: Avoiding evolutionary change can be pursued by controlling the breeding values for specific traits or, in a general way, by minimising the changes in the genetic makeup due to drift. Saura et al. (Cons. Genet. 2008) already proved that minimising coancestry (even from pedigree information) lead to the smallest change in allelic frequencies. More recently, different authors (Meuwissen et al. 2000, Front. Genet., Morales-González et al. 2021, Genes) explored the effects on the change of allelic frequencies from the use of different molecular coancestry matrices in the management of conservation programs.	Thank you, we have included these points. The link between the ancestry matrix used and maximizing genetic variation versus maintaining frequencies closer to the base population was interesting. **Line 165-170:** Minimizing mean kinship will reduce allele frequency change and depending on the kinship matrix used managers can maximize the amount genetic variation or maintain allele frequencies closer to the base population (Meuwissen et al. 2020; Morales-González; Saura et al. 2008). However, monitoring and controlling breeding values for specific traits could be combined with management plans to identify and control potential evolutionary change.
- Lines 182-183: This sentence is a bit misleading due to the expression 'contribute'. I think that something like 'affect' or 'has a great influence in the performance for important traits'. Moreover, this is also true for large populations, at least for the part of the genetic variation.	Yes, it was missing the word non-additive. We wanted to mentioned that here as a consideration for management of small population sizes. We've softened the sentence a bit. **Line 188-189:** Because of the relatively small size of captive populations, non-additive genetic variation and increased inbreeding could also contribute to the variation in traits
- Lines 195-196: I find this sentence a bit 'speculative' because in practical terms new captures to be included in ex situ populations are mainly opportunistic. It is quite difficult to pick up the individual with the required neutral variation to enlarge the genetic background of the population and it may be even harder to detect the one with the 'appropriate' breeding value.	Thank you, this is a good point. We've rewritten this section as below. **Line 205-214:** If changes in the average breeding values are determined to be of concern, managers could increase gene flow from wild populations or to drive breeding values in a desired direction through selective breeding. Increasing gene flow and selective breeding comes with difficulties and depends on sampling individuals from the wild that have breeding values that can alter the average captive breeding value in a desired direction. Knowledge of the wild population will help inform strategies that use gene flow to alleviate evolutionary change in captivity (e.g. sampling relatives from families with estimated breeding

	values in captivity). Selective breeding should be done with caution because it could reduce genetic diversity and have unintended consequences through selection on correlated traits (Ralls et al. 2000; Lande & Arnold 1983; Arnold & Wade 1984a, 1984b).
- Lines 208-209: Please, rewrite the definition of Animal Model; it seems that all individuals have to be founders with not known ancestors to apply this methodology. Additionally, there are developments of Animal Model methodology that allows for the several populations, for example in crossbreeding selection schemes where not only (additive) breeding values can be estimated but also dominant effects calculated and accounted for.	Thank you, we have restructured our discussion on genetic groups to highlight the original use more appropriately as well as note that molecular markers will potentially be an invaluable tool to inform such an analysis.

Line 224-251: Founders in a population might come from populations with different genetic backgrounds that might have traits with different average breeding values. Using genetic groups, Animal Model methodology can account for known or assumed genetic structuring in a studied population (Wolak & Reid 2017; Lacy 2012). Genetic groups are researcher defined groupings that are ideally informed by knowledge of assumed or known genetic structuring in the wild (founders from distant populations or molecular marker informed population structuring). |
- Line 212 and followings: And alternative for the genetic groups is the use of molecular information in a punctual way to estimate the genetic relationships between founders or to apply the concept of 'metafounders' in the evaluation of the genetic merit.	One valuable approach for joint *ex situ* and *in situ* management could be to assign founding individuals, and progeny produced in the first few years of a conservation breeding program to one group, and later immigrants brought into captivity as a second group. The proportion of each offspring's genome attributed to the *ex situ* versus *in situ* population can then be determined using the studbook pedigree. Beyond just accounting for biases, partitioning individuals among genetic groups in this way allows explicit measurement of the effects of wild population gene flow on an average trait value in the captive population (Wolak & Reid 2017). A difficult decision for managers will be to determine the number of genetic groups to use for a given conservation program. For example, after how much time should new individuals brought into captivity be considered a new genetic group? Analysis of molecular markers could possibly help inform the number of groups to use in a genetic group analysis. If enough data are available in the wild, trait values could also be monitored and quantified for the *in situ* population, which would provide comparisons to help
- Lines 218-219: The original idea of the concept of 'genetic groups' was to account for the fact that different founders might come from different populations (with different genetic backgrounds). For example when a single captive population is constructed by sampling individuals from several isolated regions. If new captures occurs in the same original population a not much different background is expected (in general) as to consider that is a different genetic group. Moreover, when is time to change to a new group? Two generations? Five?	
- Lines 220-221: This feature is not (completely) related to the definition or not of genetic groups. Even if all founders are assumed to be from the same group, the proportion of insitu (founders' contribution) and exsitu (non-founders' contribution) influence in the genome can be calculated from the pedigree and monitored.	

	determine the extent to which captive individuals differ from a baseline (Fig. 1). Additionally, recent advances in analytical methods allow for the measurement of different additive genetic variances between groupings and extend genetic group methods to genomic relatedness, which may be useful for comparing the adaptive potential of a trait in the wild or captive population (Muff et al. 2019; Aase et al. 2022). A study of song sparrows (*Melospiza melodia*) on Mandarte Island, Canada provides an empirical example of a genetic group model that mirrors an *ex situ* breeding program (i.e. a focal study population with measured and periodic gene flow). In this case, the analysis used a genetic group model to determine that gene flow to the island population is preventing local adaptation (Reid et al. 2020).
- Line 265: Replace 'a' by a comma.	Thank you, corrected.
- Line 265: From this moment it seems that all references to figures are wrong. Here it should be Fig. 4 instead Fig. 3, shouldn't it?	
- Line 292: As said before, I find quite difficult to detect plasticity but detect differences in genetic conformation by evaluating the differences in phenotype (Animal Model).	Thank you, we think this is true too and have tried to emphasize more of the GxE versus IxE in our plasticity discuss now. We do still think IxE is a potentially important consideration.
- Line 299: There is a missing 'of' between 'impacts' and 'captivity'.	Thank you, fixed.
- Line 308: I agree with the authors that differences in social interactions between exsitu and insitu populations are crucial to determine the success of reintroductions. At the same time I think it is the most difficult aspect to be accounted for when 'naturalising' the captive environment.	Yes agreed, perhaps something that always needs to be monitored, but that we can never "fix"?
- Line 318: In most captive breeding programs hand-reared animals are never used for reintroductions.	Thank you, our wording was a bit clumsy but we meant captive reared in general. It now reads as captive reared.
- Line 320: The correct spelling is *Callithrix* (missing 'i').	Thank you, fixed.
- Lines 335-336: Not very informative on what you will find in this section. It is plenty of lines on software.	Thank you, we've updated the section heading. 5. Putting it all together: combining quantitative genetic analyses with conservation management tools
- Line 339: Missing 'in' between 'maintained' and 'a variety'.	Thank you, fixed.

- Lines 342-343: Rewrite to avoid the use of 'options' twice.	Thank you. **Line 356-357:** Platforms provide varying options for data storage, manipulation, and export.
- Lines 350-351: It must be highlighted that (Estimated) Breeding Values are dynamic values and should be updated in the database every time a new evaluation is performed. It is not like phenotypic value for a trait (e.g. size at birth) that doesn't change even if the individual grows up and is bigger at another age.	Thank you, UDFs can be updated and could possible be used to keep track of breeding value updates. **Line 362-366:** Additionally, the commonly used studbook applications include an option to incorporate User Defined Data Fields (UDFs). UDFs can be used to record phenotypic data or quantitative genetics output such as breeding value. UDFs are flexible and can be updated which will be invaluable for estimated breeding values that will change and need to be updated every time a new analysis is conducted.
- Lines 357-360: I think that one of the main messages of the paper should be that different sources of information and different goals have to be included into the management criteria beyond the maintenance of global genetic diversity (minimisation of coancestry).	Thank you, we agree and have tried to emphasize this more in the current manuscript.
- Line 361: To be consistent with the part about the studbook and population management, some examples of QG software could be provided (for example programs to perform breeding values estimation).	Thank you, we have made slight adjustments to this sentence to better highlight this point. **Line 373-376:** Therefore, the outcomes of different gene flow, social management, and breeding strategies which incorporate quantitative genetics analyses can be modeled and considered alongside gene diversity (probability-based estimate of heterozygosity) retention and inbreeding coefficients to improve management
- Lines 363-364: This sentence is incomplete.	Thank you, fixed and combined with the next sentence to reduce redundancy.
- Line 367: It is not only the need of accuracy (e.g. pedigree completedness) but also to a standardisation of the way the measurements are taken between different facilities and across generations.	Thank you, yes this is true. We have added this in the topic sentence for this paragraph.
- Lines 372-373: This is the hard task. It would be nice to have a deeper consideration on the real applicability of these methodologies and to provide examples of cases where the situation is closer to this scenario and those where it would be nearly impossible.	Thank you, we agree. We are applying for grants to hopefully try to apply some of these methods to captive populations, we see the potential value for a few populations and hopefully can make some empirical evaluations in the future. We think the more evaluations and attempts might help improve our understanding of feasibility.

- Lines 385-386: I think that the main task conducted in current exsitu conservation programs is the priorisation of individuals to be reproduced based on their genetic relationship with the rest (before/besides the minimisation of the coancestry between pairs). This procedure acts jointly on the maintenance of genetic diversity and the minimisation of the rise of inbreeding.	Thank you, we have adjusted this sentence to make it more accurate. **Line 399-400:** … in addition to current best practices of mate-pairing based on mean kinship.
- Line 402: I agree. It is clear that studies on insitu populations are needed to know the target traits and values to be pursued in captivity. And the same goals should be established for both environments.	Thanks.
- Line 404: It is difficult to deal with fitness itself. Sometimes it is more useful to concentrate on particular traits (main components of fitness).	Thank you, in this context though, we think, it is important to try to use a metric as close to actual fitness as possible. It may be easier to do in the captive population than the wild.
- Line 405: There is an extra space before Bonnet.	Thank you, removed.
- Line 409: Something missing between 'be' and 'to'.	Thank you, the word important was added.
- Line 418: I think that the first/main aim is to reduce the loss of diversity. Minimising inbreeding is a complementary aim, which should go together. Realise that it is hard to minimise inbreeding when little genetic diversity is present.	Thank you, agreed and changed to below. **Line 430-431:** "...despite best management practices for *ex situ* populations that include efforts to reduce the loss of diversity."
- Line 664: Capitalise Fisher and Wright.	Thank you, done.
- Line 696: Provide a caption to the Figure 2. Additionally, I think that the information presented in the left and right parts of the figure are different enough to deserve separated figures.	Thank you, we have redone this figure now.
- Line 698: Is the word 'in' really needed between 'individuals' and 'might'?	Removed, thank you.
- Line 700: Change the word 'or' by 'and' because in this scenario differences in environment.	Thank you, done.