General answer:

Dear Michael Greenfield,

We have now completed our revision of our manuscript. We thanks both the recommender and the reviewers for their comments and suggestions.

We now include several references to other evolutionary genetic ABM simulation approaches and clarify our own contribution, regarding its specifics and novelty (mating systems and sexual selection).

We modified the manuscript to better describe our approach and why it could be useful for other scientists. We also shortened the paper in the process, by removing some simulation experiments, to focus in detail on the core mechanics of our model. We hope that the paper does a better job now at motivating the use of explicit demogenetic ABM approaches in the sexual selection context, something that is currently rare in many eco-evolutionary studies.

In reaction to the second reviewer's thorough analysis, and in accordance with our previous exchange, we updated the genetic description of the model to make it clear that we use a fully explicit allelic approach – as opposed to a Quantitative genetic approach. We now better explain this choice in relationship with the life history trade-offs relating traits values and survival in the model. Following again the reviewer's advice, we now refer to allelic correlations instead of pleiotropy, the latter being too general for what we investigate in our model. We also clarified our bootstrap approach for this measure. Regarding polygeny, we also followed the reviewer opinion by referring now to oligogenicity and proposing a measure to quantify the variance of genetic values between loci.

We now also address in discussion some ways to validate some parts of the model – mainly patterns – such as the speed of evolution, genetic variance in relationship to selection type, and genetic correlation between traits, for instance.

We believe the manuscript has improved from these modifications, and therefore hope that it will now meet the standards for being recommended by PCI BioEvol.

Sincerely yours,

Louise Chevalier and collaborators.
Response to reviewers’ comments:

Round #1

Major revisions needed

It is clear that this manuscript addresses an important issue in evolutionary biology and animal behavior, and one that could benefit from more modeling and simulation. However, it is also clear that the overall objectives and modeling approach of the paper need to be clarified, that an assessment of the validity of the model should be attempted, and that many genetic details need correction. Reviewer 1 has called attention to the lack of reference to other modeling approaches currently in wide use and how the manuscript's method compares with them. Reviewer 2 offers detailed suggestions for overall improvement and for sharpening the presentation of the study. All of these points will have to be addressed before a recommendation can be considered.

Michael Greenfield

Reviews


The paper presents itself as a proof-of-principle for “demogenetic agent-based models” in evolution. I am having difficulty distinguishing their approach from individual-based simulations that are widespread in the literature, like SLiM, that are somehow not cited here. I feel like they use a vocabulary that creates unnecessary distinctions and confusion with respect to most of the literature on population genomics. I am still wrapping my head around the notion of a “mean genome” The paper does not engage contemporary simulation-based work or population-genomic theory. I think it needs to be rewritten extensively to clarify the distinctions with other agent-based models and the unique contributions this approach could make, if any.

We now provide more context regarding genetic individual based model, and point out our originality, which is to 1/ fully simulate explicitly the sexual selection context, 2/ directly relate the genetic bases of traits to life history trade-offs (effect on survival).

Vocabulary issues regarding the genome context have been addressed, following reviewer 2 suggestions.

Reviewed by Frédéric Guillaume, 2020-06-08 18:52

I have now reviewed the manuscript "A demogenetic agent based model for the evolution of traits and genome architecture under sexual selection" By Chevalier et al. This work describes a simulation program to model the joint action of mating preference, mating system, and genetic architecture. The intention is to simulate the effects of sexual selection on the evolution of the mating system. Although there are many individual-based, genetically explicit evolutionary simulation software available, I am not aware of any who has implemented such models yet. The
model allows the user to address fundamental questions on evolution under sexual selection, a key evolutionary force in nature.

I have a number of concerns about the implementation of the model, and especially about the interpretation of the genetic output. I will mostly comment on the genetic architecture.

Major comments:

As said, it is one among many individual-based, forward-in-time, genetically explicit, stochastic simulation software available "on the market". Yet, the manuscript doesn't reference any of those other tools. The authors should acknowledge that diversity and position their tool relative to what new it brings and how different it is from the rest.

Indeed, we failed to make reference to other genetically explicit IBMs such as simuPOP (Peng and Kimmel, 2005) Nemo (Guillaume et al. 2006) quantiNemo,(Neuenschwander et al. 2008). We now mention it and highlight the main differences with our model: In the above mentioned models, the selective pressure on traits is a priori defined and therefore do not emerge from inter-individual interactions, and from trade-off between traits.

By contrast, in our approach, traits values directly influence mating behaviors and survival of individuals, allowing the selection pressure to change with the distribution of the traits values in the population; organisms thus modify their social environment and are able to respond dynamically to this change. Additionally, to our knowledge none of these models particularly emphasizes the general role of sexual selection as a driver of traits and genetic architecture, whereas we explicitly model competition and preference interactions. Finally, these genetic bases are directly related to life history-tradeoffs in our model.

(My feeling is that the simulation software is designed to address specific questions in a specific context with a specific model. The general approach (demogenetic agent-based simulations = eco-evolutionary individual-based simulations) is well developed in other more advanced and flexible simulators. The audience and use of the software is thus likely to remain specific as well. Which is fine of course.)

The manuscript is part a tool description and part a model description. While it is not always easy to clearly separate the two aspects of the approach, the authors should focus on one of the two aspects. If the goal is to propose a new, cool simulation software, then less room should be dedicated to the description and interpretation of the results of the simulations. The manuscript would then greatly gain in readability.

We followed the reviewers suggestions, by describing in more depth the use and the interest of the model, and we removed some exploratory results, to better focus on the core mechanics in the model.
I am missing information about how users can do to use that software, how they can interact with it and run simulations, etc.

We now provide a guide on a web page describing how to install, run, and manipulate the model at the following address: http://capsis.cirad.fr/capsis/help_en/runaway.

If the goal were not to "sell" a simulation software, then the focus should be made on the evolutionary question and less on the simulation approach. For now, it is a bit a hybrid between the two and might miss its audience.

One of my major criticism is that the authors did not care to validate their simulation results with known theoretical results. The results presented are of little value because we do not have a way to assess their validity. What assures us that the simulator is capable of producing reasonable outcomes based on known expectations?

Because our model is not based on quantitative genetics, explicitly describes the whole genetic bases of traits, it might not be validated in the spirit proposed by the reviewer – as for instance, a quantitative genetic based models, which is more connected to empirical description of evolution. However, the model produces patterns that can be compared to general knowledge, either theoretical or empirical. We now cover that question in discussion in a dedicated paragraph.

The rest of my comments are relative to the genetic setting and interpretation of the simulations.

>>Polygenicity:* 

LL318-323: I don't agree with the definition of the "degree of polygeny" as the number of loci of major effects. If anything, the degree of polygeny should DECREASE with the number of loci of major effects. The higher the number of loci affecting (equally) a trait, the higher the polygenicity. A polygenic trait is defined as a trait affected by many loci of small effect. What you describe is a degree of "oligo-genicity". Please better justify your choice here.

We agree with the reviewer that our approach to polygeny is somehow confusing: this is so because we do not refer to a quantitative genetics model, and therefore we do not look at the relative effects of mutations – we look at their full effect in the construction of the trait value.

We changed this by another measure, wherein we look at the standard deviation of genetic values between loci pondered by their mean value: this should indicate if all genes contribute equally of very differently to the total genetic value (i.e., trait value in our case). We refer to it as inter-loci variation in genetic value. This new approach better captures the relative effects of the mutations present in the population.
Pleiotropy:

I don't agree with the interpretation of pleiotropic and non-pleiotropic allele values here. Allele "0" is not equivalent to "neutral" because phenotypic value "0" can be selected for. The optimum trait value can be 0 (it can also be negative). Quantitative phenotypes and alleles have in principle unbounded continuous values. Thus, modularity cannot be inferred from the allelic distribution. Instead, modularity of the genotype-phenotype map is typically evaluated from the pattern of genetic covariance between traits. Traits are said independent when they are not correlated, their covariance is zero. Trait modularity is found when traits can be grouped within modules of correlated traits. However, modularity at the gene level is a function of the pleiotropic connections of the genes to the traits and here, unfortunately, the "0" allele doesn't count as an absence of pleiotropic connection.

Ok. “pleiotropy” in our case is not the fact that one locus affects one or several traits: we assume all genes have effect on all traits. From an empirical point of view, one tries to assess variation in DNA at some loci on traits expression, and if no effect is detected on a given, then one concludes that the mutation is neutral. If effects are detected on several traits, it is said to be pleiotropic. In our case, if a mutation occurs, the value of the allele can change for no trait, for one trait or several traits: the mutation itself can therefore be pleiotropic or not. The allele value in any case will always contribute to the value of the trait.

The approach mentioned to the reviewer is however based on quantitative genetics principles. We however do not describe “neutral” alleles, we just describe their respective contribution to the trait. If all alleles have a zero value for a given trait, then the trait is zero but we agree that can still be an optimum. However, we also look at the modularity in the genetic architecture, at the gene level. For instance, we want to be able to let evolve situations where traits are actually independent because loci located on some part of the genome have variable allelic values for trait A but not trait B, whereas other genes in another area of the genome have variable allelic values for trait B but not trait A. Such case is maximum modularity, with no need for zero values.

What you describe here is not what is commonly understood as the "degree of pleiotropy", but is closer to the correlation of the allelic effects. It is also unnecessarily complicated. You should clarify and justify why you use a different approach in your case. If/when genes have similar allelic values on different traits, those traits will become genetically correlated. Traditionally, this is investigated by measuring the genetic variance-covariance matrix (the G-matrix) of the traits. The genetic independence of the traits is then evaluated from the structure of the G-matrix, especially from its modularity. See discussion in Chebib, J. & Guillaume, F. (2017) What affects the predictability of evolutionary constraints using a G-matrix? The relative effects of modular pleiotropy and mutational correlation. Evolution, 71:2298-2312. Arnold, S. J.; Bürger, R.; Hohenlohe, P. A.; Ajie, B. C. & Jones, A. G. (2008) Understanding the evolution and stability of the G-matrix. Evolution, 62:2451-2461

As explained before, we are not using the quantitative genetics framework. Our objective is to go beyond the “G” matrix: we are interested by the spatial structure of allelic values along the genome.
Our bootstrap approach thus investigates modularity at the gene level. Our measure thus accounts for the mutations retained in the population versus what would be obtained by randomly sampling into the mutations distributions. It therefore describes how the evolution of the population selects for some particular arrangement of correlated allelic values for the various traits. This in turn could influence the G-matrix, that could still be calculated, but our approach does not meet the usual assumptions that are made when using the quantitative approach (i.e., Gaussian distribution of mutation effects).

We agree that “correlation of allelic effects” is a much better term to describe our approach, and we use it now in the manuscript. We explain the bootstrap approach in details.

Note that absence of genetic correlation is not synonymous of absence of pleiotropy because genes can be fully pleiotropic (affect all traits) and yet, the traits may remain uncorrelated genetically and phenotypically (like you observe in your simulations). The pleiotropic degree (the number of traits affected by a gene) is thus not informative of the correlation among traits. If you intend to keep using the index of "pleiotropic degree", you should rename it to avoid confusion with the more common use of this term.

Right, we now use the term of allelic values correlation.

L409: this not an absence of pleiotropy but an absence of correlation among allelic effects. Pleiotropy does not evolve in the model, each locus is still affecting each trait. You are confounding genetic correlation with pleiotropy. Here, the correlation among allele values at the pleiotropic loci is apparently under selection.

Corrected.

L417: referring to comments above, the emerging modular genetic architecture is caused by the evolution of the genetic covariance among traits, and not of pleiotropy itself.

Corrected.

LL582-584: it is not a new or surprising result that genetic correlation can increase without a change in pleiotropy. All is needed is an increase in the co-variation of the genotypic values of the corresponding traits. The way the allelic values are distributed within genes will not necessarily reflect on the trait correlation as long as it varies among genes, because what matters is the sum of those effects on the traits. I would thus not call pleiotropy the difference in allele values at a particular locus as it is anyway strongly dependent on mutational input, moreover when using a strongly skewed mutational effect distribution. Therefore, the pattern shown in Fig 5 is not likely to be very stable over time and likely to be idiosyncratic.

We agree that genetic correlation can arise without pleiotropy. However, the present result is in fact quite stable and robust to the various mutational distributions (uniform, skewed, bell-shaped) we explored (with respect to the difference of mutation definition in our framework versus the QG framework). For instance, in the example of the uniform distribution, the value of the trait is different and this from the initial state since the genetic values are generally higher, but the
distribution of the allelic values tend to be organized in the same way, i.e. negatively correlated compared to what could be observed by chance.

?>>Mutational effect distribution:

L136: why a Beta distribution? Justify

As previously explained, we do not implement the QG framework. Our mutational distribution can be manipulated by the user, so to have a control on the whole distribution of allelic values that contribute directly to the trait value (and not relatively to an optimum in the population). The skewed Beta distribution in the present example was selected so to start from relatively low values of traits so to ensure that the initial trade-off between traits values and survival will not crash the population, and to balance the initial conditions between the investment into reproduction and the probability to survive.

L237: what justifies your choice of Beta distribution for allelic values at your quantitative trait loci? The mutational distribution of quantitative trait loci (QTL) is typically assumed a Gaussian (multivariate Normal) and not Beta in the literature on quantitative genetics. Are you referring to the distribution of deleterious mutations? This shouldn't apply to QTL.

See answers above.


Because we are not using the QG framework, our mutations are not distributed as relative effects to the mean, so the above calculation may not be pertinent – but we might be wrong. A possible avenue would be to actually “sample” in our model, as in empirical approaches, to detect the effects of mutations around a pseudo-stable equilibrium. However, it would require to build a sampling protocol, with its own set of parameters to justify. This seems to us beyond the purpose of the present study. We do however provide some ways in a new paragraph in discussion to validate some parts of the model – mainly patterns – such as speed of evolution, genetic variance in relationship to selection type, and genetic correlation between traits for instance.

LL498-501, Figs 9-10: the difference of the evolutionary dynamics between the 10loci and 100loci architectures seems suspicious to me. Did you check that the mutational variance per trait is the same in both models? the evolutionary dynamics should be the same for a similar level of
mutational input to the variance (Va) and covariance of the traits. Because you use a non
conventional mutational distribution for quantitative loci, it is very difficult to evaluate if biases
were introduced by your modelling choice. I surmise that the difference between the 10L and 100L
cases is caused by differences in the mutational variance-covariance of the traits. You should
compare your results with simulations using a multivariate Normal distribution for the mutational
effects.

We removed this part now, so to focus on other points of interest, as suggested by the reviewer in
his preamble.

Mating groups:* LL157-158: explain why and how should the user decide to divide the population
into mating groups. What is the rational behind this modelling choice? How should the user choose
a value for M?

One of the major characteristics of sexual selection is that it is extremely context dependent.
Therefore, availability of sexual partners as well as their phenotypic and genotypic distribution are
central in the outcome of sexual selection. This is basically one of the reason we do model
interactions between individuals explicitly and why we provide this option for people interest in
the study of sexual selection. We added some details in the methods part.

L162: random mating: this procedure describes a monogamous mating system and not what is more
commonly understood as "random mating" where all gametes from all individuals mix freely (also
taking account of gender). The difference would be that, under random mating, the offspring of a
particular female are not all from the same male, in contrast to monogamy. Here, you need to clarify
how many offspring are created from each mating and whether your mating system differs from
monogamous mating.

We agree that the “random mating” routine in the model can be assimilated to “monogamous
mating” to some extent: it only lasts one breeding season, whereas, depending on the evolution of
the trade off, individuals can potentially participate to several seasons. However, it is important to
remind the readership that in this routine, pairing between mates is the result of a random process.
What you seem to call random mating – perhaps in a population genetics sense – is possibly
panmixia. From a behavioural, sexual selection point of view, these are two very different systems.
We now give this precision in the manuscript.

Regarding offspring creation from each mating: this is the average of parental gametic investment
multiplied by a demographic constant.

**Minor comments**

L13: polygyny -> polygenicity

Done.

L18: retroactions -> feedback
Done.

L105: stick to one term, either agent-based or individual-based

We now use Agent based model (ABM)

L121: The process for simulations -> The simulation process

Done.

L123: "procedures are processed" -> better call those procedures something like "life cycle events", "procedure" is more of a technical word

Done.

L125: probability of surviving for individuals -> the "individual surviving probability" or "surviving probability of individuals"

Done.

LL126,144: what are "costly traits"? clarify how these costs are expressed and set

Done.

L128: drawing "lots" of individuals -> be more specific, what and how much is "lots"

Done.

L131: replace comma with dot, start a new sentence at "Their sex..."

Done.

LL 134-135: not clear if "New alleles are assumed to be created by mutation" means something different from the statement in previous sentence. Sounds redundant.

Done.

L148: in considering -> when considering

Done.

L149: remove "individual" ? meaning unclear

Done.

LL171-172: what stops that iterative procedure?
Done.

L176: italicize "M" throughout the manuscript

LL188-189: what is meant here is not clear. What stops the sequential procedure? how many matings are processed and how is a non-effective mating affecting the subsequent ones? Indeed, we suppressed this sentence and now better explain each behavioral iterative procedures.

L191: an individual is not a "he": "his" -> "its"

Done.

L223: per loci -> per locus

Done.

LL224-225: more recent citations on mutation rates should be given. Progresses have been made in estimating mutation rates from genomic data during the last decade.

Done.

L230: Alleles effects -> Allelic effects

Done.

LL251,253: use gender neutrality for "individual": "he" -> "it" (or feminise them, for a change)

Done.

LL273-280: make extra clear that the costs mentioned are all implicit, no parameter allows the user to set those costs explicitly

Done.

L284: the purpose of these comments isn't clear, are they necessary? This comment aimed to point out at the difference between our approach and behavioral ecology approach (where the behavior is directly optimized). We change the section thought to make our point clearer.

L287: not clear what you mean with "relative to other strategies". It should be strategies of the other individuals

Done.

L292: first time the extrinsic environment is explicitly mentioned but without details. How is that environment set? Specify
It is the resource limitation $K$ as explained in Eq.1

L294: gender neutrality!

Done.

L366: what conditions are necessary for a pseudo-equilibrium versus an equilibrium? the distinction should be clarified for the rest of the examples. We rewrote this part

L416: not clear what a mutational landscape is. You should refer to the distribution of mutational effect for clarity. In fact we simply meant “allelic values distribution”

Author’s Reply:

Comment of the managing board: the following is a direct answer of the authors to one of the reviewer.

Dear Dr. Guillaume.

First and foremost, thank you for this detailed review of our manuscript, and all the work accomplished. We apologize for the delay in our response. We plan to resubmit an improved version of the document to PCI Evol Biol in the coming month. We believe the paper can be significantly improved through clarification, following your advice, in a few general directions, and we would like to have your opinion on the following choices:

- First, by better positioning our work: As noted, we failed to position our paper between other genetically explicit IBMs, the main particularity of our model is the focus on behavioral interactions between individuals in the context of sexual selection. In other genetically explicit models, the selective pressure on traits is a priori defined and therefore do not emerge from inter-individual interactions, and from the trade-off between traits. By contrast, in our approach, traits values directly influence mating behaviors and survival of individuals, allowing the selection pressure to change with the distribution of the traits values in the population, organisms thus modifying their social environment and being able to respond dynamically to this change.

- Second, by stating more clearly our genetic formalism: Indeed, we noticed that you interpreted several aspects of our model following a quantitative genetics point of view. Our model is not based on quantitative genetics, and therefore several of your comments do not apply. Still, the fault is ours for not having described more clearly the genetic approach (allelic model). There is indeed no neutral mutation (or allele) in our model, and the genetic value of zero for an allele has an effect on the trait(s) expressed. This choice is motivated by the fact that our model directly integrates a trade-off, wherein the size of the trait has a cost on survival (so in terms of natural selection). Additionally, we used the Beta
distribution to let the user simulate and approximate different possible distribution of effects for mutations: uniform distribution, biased distribution toward small or large mutations (at least relative to survival), or possibly a Gaussian-like distribution. Besides, we should have clarified our measures of genetic architecture ( "polygeny" and "pleiotropy," see further).

- We also are currently investigating how we could compare the output of our model (accounting for genetic variance, population stability – or the lack thereof – effective number of breeders) to theoretical expectation under the assumptions of quantitative genetic (Turelli, 1984; Burger, 2000) as you suggested. Namely, we want to investigate the relationship between the effective number of breeders in the population, the selection regime and strength, and the genetic variance. To do so, we will focus on the random mating scenario (no preference no competition) and compare the genetic variance of gametic investment with theoretical expectation (by approximating the effective number of breeders in the population, the selection regime, and strength). We hope this example can answer your concern.

- Lastly, we noted that the manuscript appeared as ambivalent (between the description of a modelling approach and analysis of evolutionary situations), and we agree that it should somehow be rebalanced. Our opinion is that the new version of the manuscript should indeed focus more on the presentation of the model and on its perks/interests/benefits compared to the existing literature. Our general philosophy would therefore to strip the paper of part of the analyses (regarding the effect of physical structure of the genome on the evolution), re-write some portions of the presentation (namely how to install, launch and handle the simulations), and include a paragraph dedicated to the specifics of our approach in relation to existing models.

- We noted your concern regarding our vision of the genetic architecture. We agree that what we termed as “polygeny” was in fact a description of the distribution of effect sizes, and that we could call it “oligogenicity”: to go further in that direction, we are currently integrating a measure of the variance between loci (centered on the mean of the population), so to distinguish simulations where all genes have approximately the same contribution to the trait value versus simulations where loci contribute unequally to the trait value. This, for us, is of interest, because it shed some light on how natural and sexual selection shapes the contribution of various loci to reach an evolutionary pseudo-equilibrium. Regarding “pleiotropy” in our model, we acknowledge that it can be more accurately called “correlation of allelic effects”. However, we stress the interest of looking at this correlation at the gene level, because it can impact the evolution beyond what it can be predicted by the G-matrix. The G-matrix sums up all necessary information to describe correlation between traits at condition that the distribution of allele effects is normally spread around mean values, and that traits are coded by an infinitesimal number of loci with small additive effects (which is not mandatory in our model). By contrast we here propose to study the spatial organisation of allelic values in the genome, and therefore investigate pattern of correlation at the gene level. Our bootstrap approach accounts for the mutations retained in the population versus what would be obtained by randomly sampling into the mutations distributions. It therefore describes how the evolution of the population selects for some particular arrangement of correlated allelic values for the various traits. Indeed, the association of allelic values for the different traits influence the number of combinations to produce given values of traits, and thus influences the conjoint transmission of traits and the variance of genotypes in the population. This is demonstrated by the results showing that these bootstrap values converge - depending on the simulation scenario - towards
specific values, this pointing at a particular organisation of correlated allelic values with respect to the spatial structure of the genome (Figure 8). Additionally, the model also calculates such measure at the individual level to observe how the correlation of allelic effect is related to fitness (Figure B3, supplementary material).

We hope to have clarified our approach and its interest, and we would welcome your feedback. We believe this exchange will be valuable to us in resubmitting an improved version of the paper to PCI.

Sincerely Yours,

Louise Chevalier and Jacques Labonne.

---

**Round #2**

**by Michael D Greenfield, 2020-08-19 10:35**

Manuscript: [https://www.biorxiv.org/content/10.1101/2020.04.01.014514v1](https://www.biorxiv.org/content/10.1101/2020.04.01.014514v1) version 1

**Please revise**

**Reviews**

Reviewed by Frédéric Guillaume, 2020-08-17 08:43

I am mostly OK with those pre-revision comments and suggest that the authors revise their manuscript accordingly.

However, I am stressing that the authors should make clear how their approach it different from current approaches in other simulation software. They should also be extra careful to clarify the generality of their genetic analysis approach and the link to outstanding questions in evolutionary quantitative genetics or population genetics. This is necessary to clarify the audience of the software. I would like the authors to answer two questions: Who is going to use your product?

For theoreticians in behavioral ecology who wish to test the robustness of their predictions when adding genetics.

For populations geneticists who are aware that demographic AND behavioural interactive processes are central in the populations structures. In particular, sexual selection has profound and extremely context dependent effects, we therefore provide a tool to investigate these effects.
and for what purpose?

To me, the work described has two purposes: the individual-based simulation, and the analysis of allelic correlations with a bootstrap method. Could a user use the software to perform the analysis only? Is there interest in having such genetic analysis as a standalone method? If so, you might be better off separating the two aspects in two distinct products/publications.

We agree with the reviewer, and we add that the focus on sexual selection is for us both original and paramount: with the present model, one can actually investigate simultaneously how traits evolves in a complex mating market dynamics, with regard for population dynamics, and the inclusion of the genetic constraint as well as the feedback of sexual selection on trait and genetic bases evolution. That is why it makes not really sense to separate these components into different packages. We might, if requested by some user, provide the code for the bootstrap analysis.

About the statistical method. Please clarify how this is different from analysis of pattern of linkage disequilibrium (LD) along the genome.

We are not sure how the two approaches compare