# **Title:** How to incorporate information on propagule pressure in the analysis of alien establishment success

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## Abstract

1. Identifying the factors that determine the success of biological invasions has major consequences for both ecological theory and conservation decision-making. Reliably inferring these factors depends on adequately accounting for the known effects of propagule pressure on establishment success, but detailed information on the size and number of introduced populations is often lacking.
2. Here, we conduct simulations to explore the effects of incomplete knowledge of propagule pressure on inferences regarding the correlates of establishment success. We compare situations where we have complete information on propagule number and propagule size across species, allowing success to be modelled at the population scale (population-level analysis), with those where data for propagule size (species-level analysis) and both propagule size and number (location-level analysis) are unavailable. We assess the ability to correctly infer the effects of a covariate on establishment success when this covariate exhibits varying degrees of correlation with propagule pressure.
3. We show that when establishment success is modelled at the level of species, rather than populations, this leads to an elevated tendency to incorrectly infer the effects of species level traits on establishment success (higher Type-1 error and lower power), particular when traits in question are strongly associated with propagule pressure. These biases are magnified when using proxies for propagule pressure, such as the number of locations where species have been introduced, and are magnified further when these proxies are converted to binary variables.

4. Our results validate the current best practice for the analysis of establishment success in alien species at the population-level, when the effects of propagule size and a covariate predicting establishment probability can both be reliably inferred. However, given the growing interest in understanding correlates of biological invasions, they strongly urge caution in interpreting results based on incomplete knowledge of propagule pressure.

**Running Head:** How to analyse effects of propagule pressure

## Introduction

A key step in understanding the process of biological invasion by alien species is to identify the factors that determine whether or not a set of individuals introduced to a novel area will establish a viable population there. Initial attempts to address this issue largely focussed on the characteristics of the species introduced, or of the location of introduction, following the priorities identified by the SCOPE program of the 1970s and 1980s (Williamson *et al*., 1986; Drake *et al*., 1989). Unfortunately, these efforts met with limited success, or at least found few consistent determinants of establishment success (Ehrlich, 1989; Vermeij, 1996; Mack *et al.,* 2000). The lack of progress was such that a major review of invasion biology in 1996 by Mark Williamson started out with the observation that the reasons for success and failure of different alien species introductions were still poorly understood (Williamson, 1996, p.2).

This situation began to change towards the end of the twentieth century, with the general realisation that establishment success was a characteristic of a population. This had two important consequences. First, analyses started to include factors that varied at the level of population, and therefore could vary between the different introduction events of a given species to a given location. The classic example of such a factor is founding population size—often termed 'propagule size' ($N_{ind}$, see Table 1 for a description of terms)—which is now known to be consistently and strongly associated with establishment success (Lockwood, Cassey & Blackburn, 2005; Hayes & Barry, 2008; Simberloff, 2009; Cassey, Delean, Lockwood, Sadowski & Blackburn, unpub. ms). Small founding populations are highly likely to fail to establish for stochastic reasons regardless of the species or location involved (Duncan, Blackburn, Rossinelli & Bacher, 2014), and this stochasticity can therefore mask deterministic drivers of establishment (at the population, species, or location level).

Second, studies started to analyse success or failure at the level of individual populations. Prior to this, most analyses had routinely analysed alien establishment success at the species level (e.g. see chapters in Drake *et al.,* 1989). This is important, because establishment is a population-level process (Colautti & MacIsaac, 2004; Lockwood *et al.*, 2005), and so analysis at the species level may be misleading (Cassey *et al.*, 2004a). To see why, consider the situation where establishment success is stochastic: whether or not an alien population introduced to a location survives is a chance event with probability, $P_{pop}$, which is not determined by any characteristic of the species or environment. Populations will not differ in their likelihood of establishment. Nevertheless, the probability that a species will have an established alien population, $P_{sp}$, will differ if species vary in the number of times they have been introduced, $N_{pop}$ (Veltman, Nee & Crawley 1996), what is often termed 'propagule number'. Thus:

$$P_{sp} = 1 - (1 - P_{pop})^{N_{pop}}$$

Assuming $P_{pop}$ is a constant, establishment success at the species level ($P_{sp}$) is then a positive function of $N_{pop}$; this is analogous to the expression for the probability that a population will establish assuming independent establishment outcomes for individuals in the population (see Leung, Drake & Lodge, 2004; Jerde & Lewis, 2007; Duncan *et al.,* 2014). Thus, a species is more likely to be classified as successful if it has been introduced more often. While a species can be introduced multiple times to the same location, this is also likely to lead to a positive correlation between establishment success and the number of separate locations to which a species has been introduced.

A further reason why analysis at the species level may be misleading is that species-level establishment success may be a function of any trait that causes some species to be introduced more often (i.e. to have higher $N_{pop}$), regardless of whether that trait has any direct effect on population establishment success ($P_{pop}$). It is well known that species with certain characteristics are more likely to be introduced (Duncan, 1997). For example, parrots (Aves: Psittaciformes) are more likely to have been introduced if they have large population sizes and wide geographic distributions, have broad diets, sexually dichromatic plumage and large body size (Cassey *et al*., 2004b). Introduced amphibian and reptile species have larger clutches and longer reproductive lifespans, while introduced amphibian species are also smaller-bodied, compared to species that have not been introduced (Allen, Street & Capellini, 2017). If such traits also affect the number of times different species have been introduced ($N_{pop}$), then we would expect them to be related to establishment success in species-level analyses ($P_{sp}$), even if they do not in any way influence population establishment success ($P_{pop}$). A similar problem is also likely to apply to any traits that happen to co-vary with propagule size ($N_{ind}$), or the total number of individuals across all introduction events, what has been termed ‘propagule pressure’ ($N_{Tot\ ind}$).

An example of how failing to include information on propagule pressure can lead to inconsistent and mistaken inferences regarding the causes of establishment success is provided by Cassey *et al*. (2004a). They found that propagule size ($N_{ind}$) in alien bird introductions is significantly related to body mass, geographic range size, annual fecundity, diet generalism, migratory tendency, the latitudinal difference between donor and recipient regions and whether or not an introduction is to an island (Cassey *et al*. 2004a). These traits are likely to be associated with propagule size ($N_{ind}$) because they influence the likelihood that individuals of a species are translocated, but they may have no direct effect on the likelihood that translocated individuals establish a viable population ($P_{pop}$). Indeed, Cassey *et al*. (2004a) showed that a minimum adequate model for global bird establishment success included only propagule size and habitat generalism; however, the same analysis repeated excluding propagule size found significant effects of geographical range size, introductions to islands, migratory tendency and the latitudinal difference between donor and recipient regions. These are all variables that have been shown elsewhere to relate to establishment success (e.g. Newsome & Noble, 1986; Williamson, 1996; Kolar & Lodge, 2001), but such relationships may be Type-1 errors in the absence of data on founding population size. It follows that data on founding population size are likely to be needed to enable genuine determinants of establishment success to be identified.

Although species-level analyses of alien establishment success fell out favour in the last century, studies inferring causes of establishment, using data aggregated at this scale, have started to re-appear in the literature. For example, Peoples & Goforth (2017) identified the number of introduction events as the most important classifier of establishment success for vertebrate species exchanged between Europe and North America, building on an earlier analysis at this scale by Jeschke & Strayer (2006). Allen *et al.* (2017) concluded that fast life history traits are associated with whether reptiles and amphibian species establish viable alien populations, and also identified a measure of propagule pressure – in this case, the number of separate locations to which a species had been introduced ($N_{loc}$), which they term 'introduction effort' (following Blackburn & Duncan (2001), although note that the definitions are different) – as a determinant of both establishment success and likelihood of spread.

We suspect that a return to species-level analysis of establishment success is motivated by the increasing availability of data on alien species distributions, coupled with a well-intentioned desire to assess the determinants of success, their generality and variation, across different taxa, but with a lack of population-level data on propagule pressure (or size or number) for most taxonomic groups (see also Bradie, Chivers & Leung, 2013; Bradie & Leung, 2013). However, these laudable aims will not be advanced if species-level analyses are misleading. That said, as far as we aware, no study has ever formally explored the consequences of analysing the population-level process of establishment success at the level of species. Thus, we currently lack firm evidence on which to base recommendations for future analysis of establishment success, or on the circumstances in which species-level approaches are more or less misleading.

Here, we begin to fill this significant gap in the literature on biological invasions by presenting simulations to explore formally how analysing establishment success using incomplete information on propagule pressure might affect our understanding of determinants of the invasion process. Our aim is accurately to simulate the process of establishment, whereby the introduction of varying numbers of individuals across different numbers of introduction events leads to the establishment of new populations, with the likelihood that any given population succeeds being a realistic positive function of founding population size. These simulations thus correspond to the ideal scenario in which we have complete information on both propagule number and propagule size across species, allowing success to be modelled at the population scale (Figure 1a). We then proceed to degrade the information on propagule pressure in various ways. First, we explore the scenario in which information on propagule size is not available, and where establishment success is therefore modelled at the species level, including propagule number as a covariate in the statistical model (Figure 1b). Second, we examine the situation in which information on propagule number is also unavailable, and where the number of locations where species have been introduced (i.e. introduction effort) is instead used as a proxy for propagule pressure (Figure 1c). For each of these situations, we assess the ability correctly to infer the effects of a covariate (e.g. species level trait) on establishment success, when this covariate exhibits varying degrees of correlation with propagule pressure. We show that the probability of finding spurious relationships between establishment success and a covariate depends strongly on the quality of data on propagule pressure, and on the strength of the correlation between the species level covariate and propagule pressure.

## Materials and Methods

### *Simulations*

We simulated the consequences of analysing the relationship between a population-level process (establishment success or failure) and a species level trait (e.g. a life history variable), while statistically controlling for propagule pressure in different ways. For each of $n$ = 100 species ($N_{sp}$), we generated the total number of individuals introduced per species ($N_{Tot\ ind.}$ i.e. propagule pressure) as a random variable drawn from an exponential distribution (rate parameter = 0.01). Values were rounded up to the nearest integer giving a mean propagule pressure per species $(\overline{N}_{Tot\ ind}) = 100$ (Figure 1d). For each species, the $N_{Tot\ ind}$ individuals were randomly divided amongst $N_{pop}$ introduction events according to a broken-stick distribution. Throughout, we term the number of introduction events ($N_{pop}$) the 'propagule number', and the number of individuals per event ($N_{ind}$) the 'propagule size' (following Lockwood *et al.,* 2005). Propagule number for the $i^{th}$ species ($N_{pop_i}$), was drawn at random from a beta distribution scaled by $N_{Tot\ ind_i}$ and rounded up to the nearest integer,

$$N_{pop_i} = \lceil B(\alpha_1, \beta_1) \times N_{Tot\ ind_i} \rceil$$

The shape parameters for the beta distribution ($\alpha_1$ = 1 and $\beta_1$ = 9) were chosen to give a mean propagule number $(\overline{N}_{pop}) = 10$ (Figure 1e) and mean propagule size $(\overline{N}_{ind}) = 10$ (Figure 1f).

In our simulation, the likelihood of population establishment ($P_{pop}$) was modelled as a function of propagule size ($N_{ind}$) according to a Weibull function,

$$P_{pop} = 1 - e^{-(\omega N_{ind})^{c}}$$

where $c$ is a constant that determines the shape of the functional response and $\omega$ is a species-specific trait that determines the mean establishment probability (Figure 1g,h). When $c > 1$, the relationship between $P_{pop}$ and $N_{ind}$ is sigmoidal. When $c < 1$, $P_{pop}$ is a positive but decelerating function of $N_{ind}$. Here we set $c = 0.5$, to approximate the relationship between $P_{pop}$ and $N_{ind}$ that has previously been described for birds (Duncan *et al.*, 2014) (Figure 1h). Values of $\omega$ for each species were drawn at random from a beta distribution with shape parameters $\alpha_2$ and $\beta_2$,

$$\omega = B(\alpha_2, \beta_2)$$

Shape parameters ($\alpha_2 = 2$ and $\beta_2 = 200$, Figure 1g) were chosen to give a mean expected probability of establishment ($\bar{P}_{pop}$) $\approx 0.2$ (Figure 1i). For each introduction event per species, we recorded establishment success (1) or failure (0) as a binary variable. At the species level, establishment success or failure occurs when at least one (1) or no (0) introduction event successfully establishes, respectively.

To assess the effects of using the number of unique locations ($N_{loc}$) rather than propagule number ($N_{pop}$) as a metric of propagule pressure (throughout we term this location based proxy 'introduction effort'; c.f. Allen *et al.,* 2017), we randomly assigned each of the $N_{pop}$ introduction events to one of 10 possible geographic locations ($maxN_{loc} = 10$). Note, not all locations necessarily receive introductions, especially when the number of species ($N_{spec}$) or introduction events per species ($N_{pop}$) is low (when $\overline{N}_{pop} = 10, \overline{N}_{loc} = 6.5$). We scored establishment success or failure at the level of locations when at least one (1) or no (0) introduction events to that location successfully established (Figure 1c). In our baseline simulation, we assume a sample size of $N_{spec} = 100$ and a mean ratio of propagule number ($N_{pop}$) to the maximum number of geographic locations ($maxN_{loc}$) of 1. However, we also explore the effects on model inferences of using different sample sizes ($N_{spec}$ = 50, 100, 400), mean propagule pressure per species ($\overline{N}_{Tot\ ind}$ = 100, 200, 1000 individuals), and number of geographic locations available for introduction ($maxN_{loc}$ = 10, 100, 500).

We evaluated the power of different modelling approaches correctly to infer the effects of ω by including this as a term in a model predicting establishment success (quantified at the level of either population, location or species).
A variety of traits may correlate with propagule pressure (see e.g. Cassey *et al.*, 2004a), because they influence the number of individuals or populations that are translocated, but these traits may have no direct effect on the probability of population establishment. To describe this scenario, we next generated a species level trait, λ, that is independent of ω but is correlated with propagule pressure,

$$\lambda = \log N_{Tot\ ind} + x$$

where $x$ is a random deviate drawn from a normal distribution with mean of 0 and variance of σ. To examine how the strength of the correlation between the species trait λ and propagule pressure influences model performance we varied values of σ (σ = 0.1, 2, 5 ,15), with smaller values generating stronger correlations. Throughout, we report the correlation between the species trait λ and either the propagule number ($N_{pop}$) or number of introduction locations ($N_{loc}$), as this is typically the only information widely available across species and is thus the basis of most proxies for propagule pressure.

*Modelling establishment success*

For each combination of parameter values, we conducted 100 repeat simulations. The output of each simulation consisted of i) the propagule size ($N_{ind}$) for each of $N_{pop}$ introduction events per species, ii) the success (1) or failure (0) of each introduction event, iii) the number of locations ($N_{loc}$) to which each species was introduced, iv) the success (1) of failure (0) of each species at each introduction location, v) the success (1) or failure (0) of each introduced species, and vi) the values of the species-level traits determining population establishment probability (ω) and propagule pressure (λ). To these data we fitted 6 different statistical models predicting establishment success either at the level of population, location or species. For each model, we calculated the Type-1 error rate as the percentage of cases where we incorrectly detected a significant association between establishment success and the species trait λ, where λ does not directly influence the likelihood of population establishment ($P_{pop}$) but is positively correlated with the propagule pressure ($N_{Tot\ ind}$). We calculated ‘power’, as the percentage of cases where we correctly detected a significant association between establishment success and ω, where ω is positively correlated with the likelihood of population establishment ($P_{pop}$) but is independent of propagule pressure ($N_{Tot\ ind}$). The six different models are as follows:

Model 1: We modelled population-level establishment success (0 or 1) as a function of the species trait (ω or λ) and propagule size ($N_{ind}$) by fitting a generalised linear mixed model (GLMM) with a random effect for species. Mixed models were fitted using the R package 'lme4' (Bates, Maechler, Bolker & Walker, 2015). In all models, we assume a binomial error structure. Model 1 represents the situation where we have perfect knowledge of propagule pressure (i.e. number and size) and analyse the relationship between the species trait and establishment success at the population level. Formally:

Model 1 <- glmer(Population(0,1) ~ log($N_{ind}$) + ω + (1|Species),
family="binomial")
or
Model 1 <- glmer(Population (0,1) ~ log($N_{ind}$) + λ + (1|Species),
family="binomial")

Model 2: As in Model 1 but establishment success (0 or 1) was quantified at the level of introduction locations rather than populations and without information on propagule size ($N_{ind}$). If at least one population in a geographic location established, this location was recorded as a successful introduction (1). If no populations introduced to a geographic location established, this species was recorded as an unsuccessful introduction (0) at that location. Formally:

Model 2 <- glmer(Location (0,1) ~ $\omega$ + (1|Species), family="binomial")

or

Model 2 <- glmer(Location (0,1) ~ $\lambda$ + (1|Species), family="binomial")

Model 3: We modelled establishment success at the species level as a function of the species trait ($\omega$ or $\lambda$) by fitting a generalised linear model (GLM), with propagule number ($N_{pop}$) included as a covariate. If at least one population in a species established, this species was recorded as a successful introduction (1). If no populations within a species established, this species was recorded as an unsuccessful introduction (0). Formally:

Model 3 <- glm(Species (0,1) ~ log($N_{pop}$) + $\omega$, family="binomial")

or

Model 3 <- glm(Species (0,1) ~ log($N_{pop}$) + $\lambda$, family="binomial")

Model 4: As in Model 3, but the number of introduction locations ($N_{loc}$) was included as a covariate. Formally:

Model 4 <- glm(Species (0,1) ~ log($N_{loc}$) + $\omega$, family="binomial")

or

Model 4 <- glm(Species (0,1) ~ log($N_{loc}$) + $\lambda$, family="binomial")

Model 5: The distribution of propagule pressure across species is likely generally to be right skewed, with many species introduced in small numbers and a smaller number of species introduced in large numbers (see e.g. Fig. 3.3 in

Blackburn, Lockwood & Cassey, 2009; Fig. 3 in Duncan *et al.*, 2014). In some cases, transforming metrics of propagule pressure in order to satisfy the statistical assumptions of normally distributed model errors may not be possible. For instance, the number of introduction locations per species analysed in Allen *et al.* (2017) was strongly right skewed, and so Allen *et al.* converted this variable to a binary metric by categorising species as either high (1) or low (0) according to the median number of locations observed across species. To investigate the effects of this practice on model inference, we here repeated this procedure for propagule number ($N_{pop}$) and fitted a GLM with the species trait (ω or λ) and binary propagule number as predictors. Formally:

Model 5 <- glm(Species (0,1) ~ log($N_{pop\_binary}$) + ω, family="binomial")
or
Model 5 <- glm(Species (0,1) ~ log($N_{pop\_binary}$) + λ, family="binomial")

Model 6: As in Model 5 but the number of introduction locations ($N_{loc}$) was used following conversion to a binary metric. This model represents the approach used in Allen *et al.* (2017). Formally:

Model 6 <- glm(Species (0,1) ~ log($N_{loc\_binary}$) + ω, family="binomial")
or
Model 6 <- glm(Species (0,1) ~ log($N_{loc\_binary}$) + λ, family="binomial")

All simulations and analyses were conducted using R version 3.2.2 (R Core Team, 2016).

## Results

The Type-1 error rates and power obtained from the six different statistical models for the relationship between a species level trait (ω or λ) and establishment success are shown in Figures 2 and 3; each plot shows the effects of different magnitudes of correlation between the measure of propagule pressure ($N_{pop}$ or $N_{loc}$) and the species level trait (λ) (i.e. different values of σ) on Type-1 error rate and power. When we have perfect knowledge of propagule number ($N_{pop}$) and analyse the relationship between the species trait (ω or λ) and establishment success at the population level including propagule size ($N_{ind}$) as a covariate (Model 1), the simulations return the expected Type-1 error rate of *circa* 5% and high power (Figure 2a). This high reliability was true regardless of the strength of correlation between propagule pressure and the species trait (λ). As expected, the power to detect the effect of a trait (ω) on establishment probability depends on sample size, increasing with the number of species ($N_{spec}$) for which information on introductions is available, with power generally >95% for 200 species or more (Figure 2a).

A model in which establishment success is analysed at the species level, with propagule number ($N_{pop}$) and the species trait (ω) included as covariates (Model 3), is associated with a substantial reduction in power compared to a population level analysis (Figure 2c). In addition, when analysing the relationship between species establishment success and a species trait unrelated to establishment probability (λ), Type-1 error rates are dramatically inflated, especially when this trait (λ) is correlated with propagule number ($N_{pop}$). Perhaps unexpectedly, we note that this bias arises even though propagule number ($N_{pop}$) is included as a predictor of establishment success in the model (Model 3, Figure 2c).

A species level model incorporating propagule number ($N_{pop}$) as a binary variable (low or high) further inflates the likelihood of false positive relationships between the species trait ($\lambda$) and establishment success (Model 5). The magnitude of Type-1 error rates increases strongly with the strength of the correlation between the species trait ($\lambda$) and propagule number ($N_{pop}$), and also increases with the number of species ($N_{sp}$) analysed (Figure 2e).

If we do not have perfect knowledge of propagule number ($N_{pop}$) or size ($N_{ind}$), but instead quantify establishment at the level of geographic locations, Type-1 error rates increase across all models (Figure 2b, d, f). This is true regardless of whether establishment is also modelled at the level of locations (Model 2) or whether it is modelled at the level of species, including the number of introduction locations ($N_{loc}$) as a predictor in the model (Model 4). Although Type-1 error rates appear higher for models analysing success at the location (Model 2, Figure 2b) rather than the species level (Model 4, Figure 2d), the latter is also accompanied by a reduction in power (Figure 2d). Type-1 error rates are similarly high in species level models treating number of introduction locations as a binary variable (Model 6, Figure 2f). As expected, power increases with the number of analysed species ($N_{sp}$) across models, but this is also accompanied by an increase in Type-1 error rates (Figure 2).

When establishment is quantified using introduction locations rather than populations (Models 2, 4 and 6), the magnitude of Type-1 error rates depends on the number of geographic locations ($N_{loc}$) to which species have been introduced (Figure 3). Type-1 error rates are elevated at all except the lowest correlations between propagule number and the trait. They are lowest when the number of locations ($N_{loc}$) is approximately equivalent to the mean propagule number ($\overline{N}_{pop}$), but increase substantially when the mean propagule number per species exceeds the number of geographic locations (Figure 3). This effect is evident regardless of whether establishment success is analysed at the level of locations (Model 2, Figure 3a) or species (Model4, Figure 3b,c), and whether number of geographic locations ($N_{loc}$) is treated as a continuous (Model 4, Figure 3b) or binary predictor (Model 6, Figure 3c) of establishment.

When establishment success, recorded at the level of introduced populations, is modelled as a function of propagule size, the positive effects of propagule size on success can be reliably detected, regardless of the correlation between propagule pressure and the species covariate ($\lambda$) (Model 1, Figure 4a). In contrast, when establishment success is modelled at the species level, the power to detect the effects of propagule number (Models 3 and 5) or introduction effort (Models 4 and 6) in a multivariate model including the effects of $\lambda$, depends on the correlation between $\lambda$ and propagule pressure (i.e. propagule number or introduction effort) (Models 3-6, Figure 4b-e). When $\lambda$ and propagule pressure are strongly correlated the effect of propagule pressure in a multivariate model is rarely significant (Figure 4b-e). As expected, the power to detect the effects of propagule pressure in a univariate model (i.e. excluding $\lambda$) is unaltered by the correlation between λ and propagule pressure (Figure 4b-e). Overall, a model including the effects of propagule number or introduction effort receives higher support than a model including only the effects of λ (Figure S1). These results indicate that the primary risk of modelling establishment success at the species level is incorrectly to infer the effects of a trait on establishment success rather than incorrectly rejecting the effects of propagule pressure.

## Discussion

Our simulations provide a realistic model of the process of establishment by alien populations, whereby the introduction of varying numbers of individuals to varying numbers of locations leads to the establishment of new populations, with establishment success being a realistic positive function of founding population size. When establishment success is recorded and modelled at the level of introduced populations, with propagule size included as a covariate (Model 1), the effects of propagule size and a species trait predicting establishment probability (ω) can both be reliably inferred (i.e. Type-1 error rate is low and power is high). Model reliability is maintained regardless of whether the trait in question is also correlated with propagule pressure, and does not depend substantially on the number of species in the analysis, unless this is small (i.e., < 100 species) (Figure 2a). This validates the current best practice for the analysis of establishment success in alien species, which reflects the fact that establishment is a population-level process (Colautti & MacIsaac, 2004; Lockwood *et al.*, 2005).

Perfect knowledge of alien introduction events is rarely available, and so real-world analyses of establishment sometimes use approximations based on success at different locations, or model success at the species level ignoring the size of each introduction event. However, both of these approaches may be problematic, leading to a higher likelihood of incorrect inference about the effects of predictor variables. We find that, if instead of analysing success at the population level, we analyse success at the level of location (Model 2), the Type-1 error rate in the effect of a species trait (λ) on establishment success is elevated. This elevation is particularly evident when propagule pressure is strongly correlated with the trait (Figure 2b), or when the number of available geographic locations is small relative to number of introduced populations (i.e. $\max N_{loc} < N_{pop}$) (Figure 3a).

To understand the reasons for this bias, we conducted further simulations to assess how the rate of establishment success for each species varies with the number of introduced populations ($N_{pop}$), where success rate is quantified as the proportion of either introduced populations or geographic locations where establishment occurs. For visual clarity, we simulated the introduction of a large number of species $\left(N_{sp} = 5000\right)$ and selected parameters to give a high expected propagule number ($\overline{N}_{pop} \approx 100$) and probability of population establishment ($\overline{P}_{pop} \approx 0.5$, $\alpha 2 = 2$, $\beta 2 = 15$). We conducted simulations that differed in the maximum number of geographic locations to which species could potentially be introduced ($maxN_{loc} = 10, 100, 500$). Within each of these simulations, propagule numbers ($N_{pop}$) vary stochastically across species, enabling us to examine how the rate of establishment success varies as a function of $N_{pop}$, when the number of geographic locations is relatively small (max$N_{loc}$ = 10), intermediate (max$N_{loc}$ = 100), or large (max$N_{loc}$ = 500) (Figure 5).

As expected, this simulation shows that when establishment is modelled at the level of introduction events, the rate of success for each species (i.e. the proportion of introduced populations that establish) is independent of the number of introduced populations ($N_{pop}$) (Figure 5a). In contrast, if establishment is recorded and modelled at the level of locations, then the rate of success increases with the number of introduced populations ($N_{pop}$) (Figure 5b-d). This is because the more populations introduced to a particular location, the more likely that at least one of these will establish. This bias is weak when there are a relatively large number of locations to which species have been introduced (max$N_{loc}$ = 500): in this case, most locations will contain only a single introduced population, and $N_{loc}$ thus converges on propagule number ($N_{pop}$) (Figure 5b). With a smaller number of locations (max$N_{loc}$ = 10), however, the chance of multiple populations being introduced to each location increases, leading to a positive relationship between propagule number and success rate (Figure 5c, d). If the species trait being analysed is correlated with propagule number then this can lead to a spurious relationship between the trait and establishment success even if in reality the trait does not influence establishment probability. This bias affects not only mixed models directly modelling establishment success at the level of locations (Model 2) but also a species-level model where the number of locations ($N_{loc}$) is treated as a covariate (Model 4).

When establishment success is modelled at the species level (Model 3), the power to detect the effects of a species trait (ω) on establishment success is reduced, particularly when the number of analysed species is small (here, when $N_{sp} < 100$). The power to detect the effects of a trait will depend on many factors besides species number ($N_{sp}$), including the mean propagule number per species ($N_{pop}$), the mean establishment probability ($P_{pop}$) and the magnitude of the trait effect (ω). More worryingly, this species level analysis also suffers from an elevated Type-1 error rate when the trait (λ) in question is correlated with propagule pressure but is independent of establishment probability. This is the case even when statistically accounting for propagule number ($N_{pop}$) by including this as an additional covariate in the analysis. This bias arises because the likelihood of establishment is known to depend on propagule size ($N_{ind}$), and failure to account for this can lead to a spurious effect of any trait statistically associated with propagule pressure.

When establishment success is quantified at the level of locations and analysed at the level of species the biases associated with these approaches are compounded. Although the Type-1 error rates associated with analysing location level establishment in a species level model (Model 4) appear to be lower than in the corresponding location level model (Model 2), this reflects the lower power of the species level model rather than a greater reliability.

When establishment success is modelled at the species level another source of Type-1 error arises when either propagule number ($N_{pop}$) (Model 5) or number of introduction locations ($N_{loc}$) (Model 6) is converted into a binary variable.

Here, the species trait λ is often erroneously found to be a significant predictor of establishment success. This is because using a binary variable decreases the amount of information captured by the proxies of propagule pressure (i.e. propagule number or introduction effort). Thus, any trait that is correlated with the true values for propagule pressure, as the species trait λ is to a greater or lesser degree in our simulations, will be a stronger predictor of establishment success, even if it is unrelated to establishment probability. This explains why the Type-1 error rate for trait λ increases strongly with the strength of its correlation to propagule pressure when either propagule number (Figure 2e) or the number of introduction locations (Figure 2f) are analysed as binary variables.

Our simulations show that the reliability of analyses of establishment success depend critically on the way the analyses are conducted, with establishment success recorded and modelled at the level of introduction events (Model 1) being the gold standard. Data to allow analyses in this way are rare, but available for some well-documented introductions, such as birds to New Zealand (Duncan, 1997; Blackburn, Prowse, Lockwood & Cassey, 2011). Even here, however, we cannot guarantee the perfect knowledge of introduction events that we need to ensure that Type-1 or Type-2 error rates are maintained at optimal levels: deeper investigations of the acclimatisation history of New Zealand is revealing more detail that suggests that available data may not be as close to Model 1 as we would like (e.g. Pipek, Pyšek & Blackburn, 2015a, 2015b). This raises the question of how good really is our understanding of determinants of establishment success?

We can be confident in the importance of propagule pressure (size or number) because this is confirmed by experimental introductions (for example, biocontrol releases), where knowledge of introductions is to all intents and purposes perfect (e.g. Memmott, Craze, Harman, Syrett & Fowler, 2005; Duncan *et al.,* 2014; Duncan, 2016). Formal quantitative meta-analysis shows that propagule pressure effects do not differ between manipulative experimental and natural experimental studies (Cassey *et al.,* unpub. ms), which further increases confidence that imperfect knowledge does not greatly bias this effect. A propagule pressure effect is also expected from basic population biology, as the probability of a natural population persisting (over some period of time) is a positive function of population size. A more open question is the extent to which factors other than propagule pressure can be reliably concluded to determine establishment success, in the absence of perfect information on introduction events, especially if those traits also influence the likelihood that a species is introduced. Clearly, analyses of variables related to establishment success will be susceptible to high levels of Type-1 error if data on propagule pressure are not included. Conversely, data on propagule pressure will need to be incorporated to allow genuine determinants of establishment success to be identified. Analyses of establishment success at the species level should be interpreted with care, particularly if success and introduction effort are modelled at the level of locations. Converting continuous data on propagule pressure (or introduction effort) to a binary variable for analysis (c.f. Allen *et al.*, 2017) should be avoided.

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## Author Contributions

ALP designed and conducted the analysis and contributed to writing and editing the paper. PC conceived the work and contributed to writing and editing the paper. TMB conceived the work, assisted in the design of the simulations and contributed to writing and editing the paper.

## Data Accessibility

No data were harmed in the production of this paper.

## References

Allen, W.L., Street, S.E. & Capellini, I. (2017) Fast life history traits promote invasion success in amphibians and reptiles. *Ecology Letters*, **20**, 222–230.

Bates, D., Maechler, M., Bolker, B. & Walker S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **67**, 1-48.

Blackburn, T.M. & Duncan, R.P. (2001) Establishment patterns of exotic birds are constrained by non-random patterns in introduction. *Journal of Biogeography*, **28**, 927-939.

Blackburn, T.M., Lockwood, J.L. & Cassey, P. (2009) *Avian Invasions. The ecology and evolution of exotic birds*. Oxford University Press, Oxford.

Blackburn, T.M., Prowse, T.A.A. Lockwood, J.L. & Cassey, P. (2011) Passerine introductions to New Zealand support a positive effect of propagule pressure on establishment success. *Biodiversity and Conservation*, **20**, 2189–2199.

Bradie, J., Chivers, C. & Leung, B. (2013) Importing risk: Quantifying the propagule pressure-establishment relationship at the pathway level. *Diversity & Distributions*, **19**, 1020–1030.

Bradie, J. & Leung, B. (2015) Pathway-level models to predict non-indigenous species establishment using propagule pressure, environmental tolerance and trait data. *Journal of Applied Ecology*, **52**, 100–109.

Cassey, P., Blackburn, T.M., Russell, G., Jones, K.E. & Lockwood, J.L. (2004b) Influences on the transport and establishment of exotic bird species: an analysis of the parrots (Psittaciformes) of the world. *Global Change Biology*, **10**, 417-426

Cassey, P., Blackburn, T.M. Sol, D., Duncan, R.P. & Lockwood, J. (2004a) Introduction effort and establishment success in birds. *Proceedings of the Royal Society, London, B,* **271**, S405-S408*.*

Cassey, P., Delean, S., Lockwood, J.L., Sadowksi, J. & Blackburn. T.M. (unpub. ms) Dissecting the null model for biological invasions: a meta-analysis of the propagule pressure effect.

Colautti, R.I. & MacIsaac, H.J. (2004) A neutral terminology to define "invasive" species. *Diversity & Distributions*, **10**, 135–141.

Drake, J.A., di Castri, F., Groves, R.H., Kruger, F.J., Rejmánek, M. & Williamson, M. (1989) *Biological invasions. A global perspective.* John Wiley & Sons, Chichester, UK.

Duncan, R.P. (1997) The role of competition and introduction effort in the success of passeriform birds introduced to New Zealand. *The American Naturalist*, **149**, 903–915.

Duncan, R.P. (2016) How propagule size and environmental suitability jointly determine establishment success: a test using dung beetle introductions. *Biological Invasions*, **18**, 985–996.

Duncan, R.P., Blackburn, T.M., Rossinelli, S. & Bacher, S. (2014) Quantifying invasion risk: the relationship between establishment probability and founding population size. *Methods in Ecology & Evolution*, **5**, 1255-1263.

Ehrlich, P.R. (1989) *Attributes of invaders and the invading processes: vertebrates. Biological invasions, a global perspective* (ed. by J.A. Drake, F. di Castri, R.H. Groves, F.J. Kruger, M. Rejmánek & M. Williamson), p. 315–328. John Wiley & Sons, Chichester, UK.

Hayes, K.R. & Barry, S.C. (2008) Are there any consistent predictors of invasion success? *Biological Invasions*, **10**, 483–506.

Jerde, C.L. & Lewis, M.A. (2007) Waiting for invasions: a framework for the arrival of nonindigenous species. *The American Naturalist*, **170**, 1–9.

Jeschke, J.M. & Strayer, D.L. (2006) Determinants of vertebrate invasion success in Europe and North America. *Global Change Biology*, **12**, 1608–1619.

Kolar, C.S. & Lodge, D.M. (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution*, **16**, 199–204.

Leung, B., Drake, J.M. & Lodge, D.M. (2004) Predicting invasions: propagule pressure and the gravity of Allee effects. *Ecology*, **85**, 1651–1660.

Lockwood, J.L., Cassey, P. & Blackburn, T.M. (2005) The role of propagule pressure in explaining species invasion. *Trends in Ecology & Evolution,* **20**, 223-228.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. & Bazzaz, F.A. (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*, **10**, 689–710.

Memmott, J., Craze, P.G., Harman, H.M., Syrett, P. & Fowler, S. V. (2005) The effect of propagule size on the invasion of an alien insect. *Journal of Animal Ecology*, **74**, 50–62.

Newsome, A.E. & Noble, I.R. (1986) *Ecological and physiological characters of invading species. Ecology of biological invasions.* (ed. by R.H. Groves & J.J. Burdon), p. 1–20. Cambridge University Press, Cambridge.

Peoples, B.K. & Goforth, R.R. (2017) The indirect role of species-level factors in biological invasions. *Global Ecology and Biogeography*, **26**, 524–532.

Pipek, P., Pyšek, P. & Blackburn, T.M. (2015a) How the Yellowhammer became a Kiwi: the history of an alien invasion revealed. *NeoBiota*, **24**, 1-31. doi: 10.3897/neobiota.24.8611

Pipek, P., Pyšek, P. & Blackburn, T.M. (2015b) A clarification of the origins of birds released by the Otago Acclimatisation Society from 1876 to 1882. *Notornis*, **62**, 105-112.

R Core Team (2016) *R: a language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria.

Simberloff, D. (2009) The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 81–102.

Vermeij, G.J. (1996) An agenda for invasion biology. *Biological Conservation*, **78**, 3–9.

Veltman, C.J., Nee, S. & Crawley, M.J. (1996) Correlates of introduction success in exotic New Zealand birds. *The American Naturalist*, **147**, 542–557.

Williamson, M. (1996) *Biological invasions.* Chapman and Hall, London.

Williamson, M.H., Brown, K.C., Holdgate, M.W., Kornberg, H., Southwood, R. & Mollison, D. (1986) The analysis and modelling of British invasions [and discussion]. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences*, **314**, 505–522.

Table 1 Glossary of terms, parameters and the range of values used in simulations.

| Term or parameter | Description | |
|---|---|---|
| $N_{sp}$ Sample size[1] | The number of species introduced | **100**, 50, 400, 5000 |
| $N_{Tot\ ind}$ Propagule pressure[1] | The total number of individuals introduced per species | **100**, 200, 1000 |
| $N_{pop}$ Propagule number[2] | The number of introduction events/populations per species | **10** |
| $N_{ind}$ Propagule size[2] | The number of individuals introduced per introduction event | **10** |
| $maxN_{loc}$ Number of geographic locations[1] | The maximum number of locations available for introduction | **10**, 100, 500 |
| $N_{loc}$ Introduction effort[2] | The number of locations where a species has been introduced | **6.5** |
| $P_{pop}$ Population establishment probability[2] | The probability that an introduction event establishes | **0.2** |
| $P_{sp}$ Species establishment probability[2] | The probability that a species establishes | **0.9** |
| $P_{loc}$ Location establishment probability[2] | The probability that a species establishes at a location | **0.3** |
| $\omega$[3] | Species trait that determines $P_{pop}$ | |
| $\lambda$[3] | Species trait correlated with $N_{Tot\ ind}$ | |
| $\sigma$[1] | Parameter determining the correlation between $\lambda$ and $N_{Tot\ ind}$ | **0.1, 0.2, 5, 15** |
| c[1] | Parameter determining the relationship between $N_{ind}$ and $P_{pop}$ | **0.5** |
| $\alpha1$[1], $\beta1$[1] | Parameters of the beta distribution determining $N_{pop}$ | $\alpha1$ = **1**, 2; $\beta1$ = **9**, 15. |
| $\alpha1$[1], $\beta2$[1] | Parameters of the beta distribution from which $\omega$ is drawn | $\alpha1$ = **2**, $\beta2$ = **200** |

Values highlighted in bold are used in the baseline simulations. [1]Primary control parameters that are directly specified. [2]Derived parameters that are determined by the primary control parameters or each other. Values are the mean expected under the baseline simulation. [3]Derived parameter values not shown for $\omega$ and $\lambda$ because these represent trait values that differ across species and for which the mean is not informative.

**Figure 1**. (a-c) The structure of our simulations modelling introduction success on the basis of propagule pressure. We model the situation where several species (circles of different colour) are introduced with differing numbers of populations (number of circles) and numbers of individuals per population (black dots in each circle) to different numbers of locations (represented here by grid squares). Within the simulations, the quality of information on propagule pressure varies from (a) complete information on both propagule number and size, to (b) complete information on propagule number but not size, to (c) only information on the number of geographic locations where species have been introduced. (d-i) expected distribution of input variables used in simulations: (d) propagule pressure per species, (e) propagule number per species, (f) propagule size per introduction event, (g) species trait determining establishment success (ω), (h) Weibull functions relating establishment success to propagule size for three exemplar values of ω, (i) the probability of establishment across introduction events. In (g) arrows indicate values used in (h). Red dashed lines indicate the mean values. The parameter values underlying each distribution are described in the Materials and Methods.

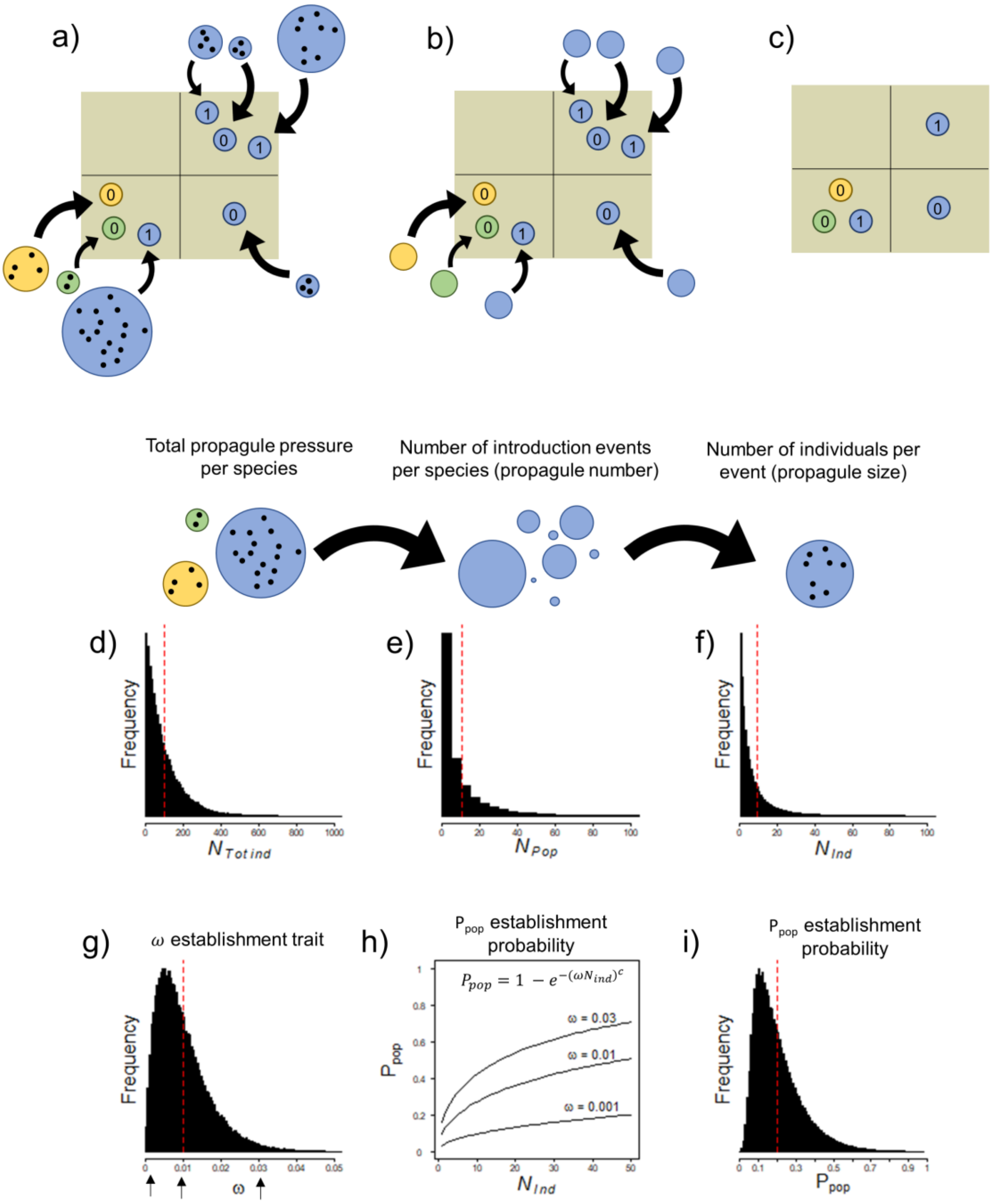
a)
b)
c)
Total propagule pressure per species
Number of introduction events per species (propagule number)
Number of individuals per event (propagule size)
d)
e)
f)
Frequency
$N_{Tot\,ind}$
$N_{Pop}$
$N_{Ind}$
g)
ω establishment trait
h)
$P_{pop}$ establishment probability
i)
$P_{pop}$ establishment probability
$P_{pop} = 1 - e^{-(\omega N_{ind})^c}$
ω = 0.03
ω = 0.01
ω = 0.001
$P_{pop}$
ω

**Figure 2**. Type-1 error rates (red) and power (blue) for models analysing the relationship between a species trait and establishment success for the six different models described in the Materials and Methods, and for different strengths of correlation between propagule number and the trait. Type-1 error rates and power are the proportion of simulations (*n* = 100 simulations) detecting a significant effect of the trait on establishment success when that effect is absent or present. Symbols indicate the number of species used in each simulation (*n* = 50, 100, 400 species).

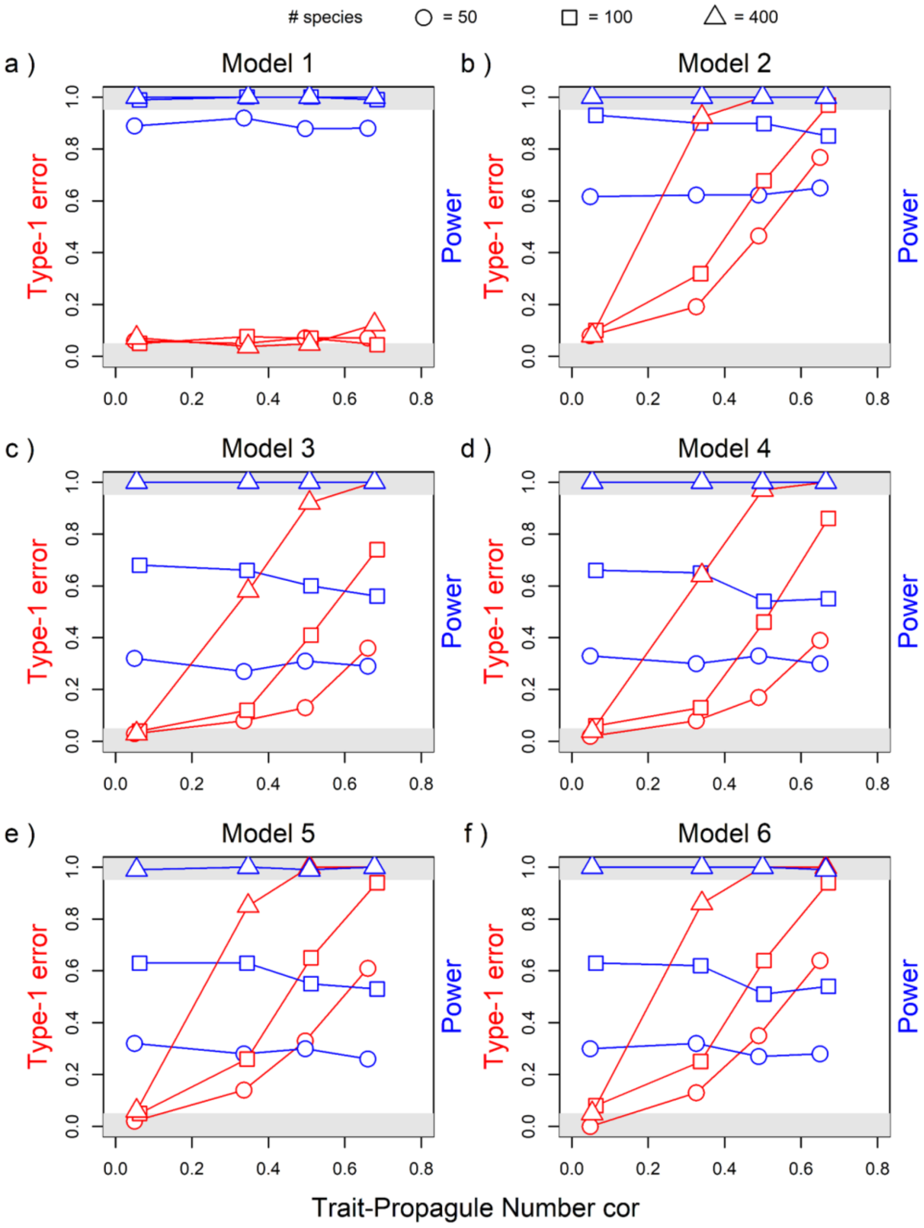

**Figure 3**. Type-1 error rates (red) and power (blue) for models analysing the relationship between a species trait and establishment success for models utilising introduction effort recorded at the level of geographic locations. Type-1 error rates and power are the proportion of simulations (*n* = 100 simulations) detecting a significant effect of the trait on establishment success when that effect is absent or present. Symbols indicate the mean number of propagules per species relative to the number of geographic locations available for introduction (propagule number/number locations = 1, 2, 10).

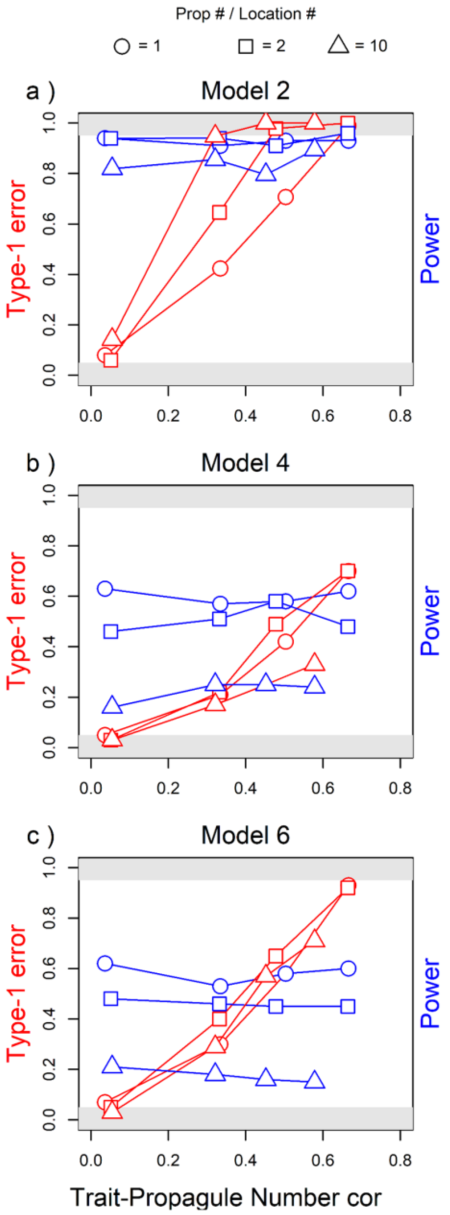

**Figure 4**. Power to detect the effects of propagule pressure on establishment success for the different models described in the Materials and Methods, and for different strengths of correlation between propagule number and the trait. Power is calculated as the proportion of simulations ($n$ = 100 simulations) correctly detecting a significant effect of propagule pressure on establishment success. Results are shown for univariate models including only propagule pressure (green) and for multivariate models including both propagule pressure and the species trait $\lambda$ (blue). The metric of propagule pressure varies across models (Model 1 = $N_{ind}$, Models 3 and 5 = $N_{pop}$, Models 4 and 6 = $N_{loc}$). Symbols indicate the number of species used in each simulation ($n$ = 50, 100, 400 species). No results are shown for Model 2 because propagule pressure is not included as a term in this model (see Materials and Methods).

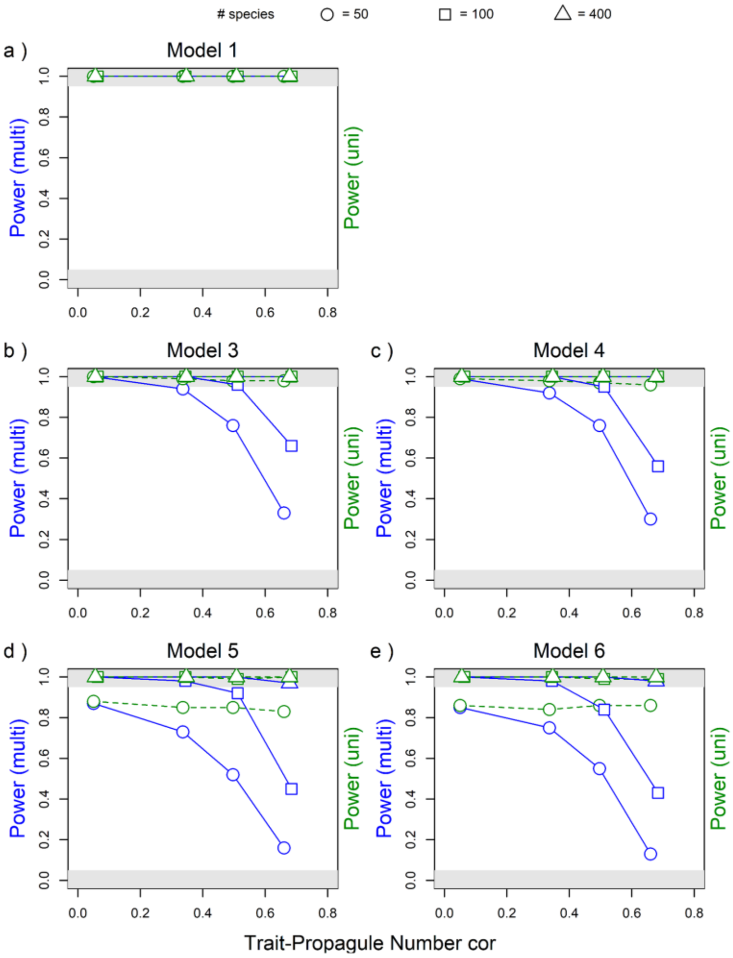

**Figure 5**. The relationship between propagule number per species ($N_{pop}$) and establishment success rate of species (i.e. proportion of events that establish) when success rate is quantified at the level of introduction events (a) or locations (b, c, d). Results are shown for cases where the number of locations ($N_{loc}$) is high (b), intermediate (c) and low (d) relative to the number of introduction events per species ($N_{pop}$). In these simulations, we used a large number of species ($N_{sp}$ = 5,000), mean propagule number ($N_{pop}$ = 100) and intermediate population establishment probability ($P_{pop}$ 0.5) in order better to visualise the expected trends.

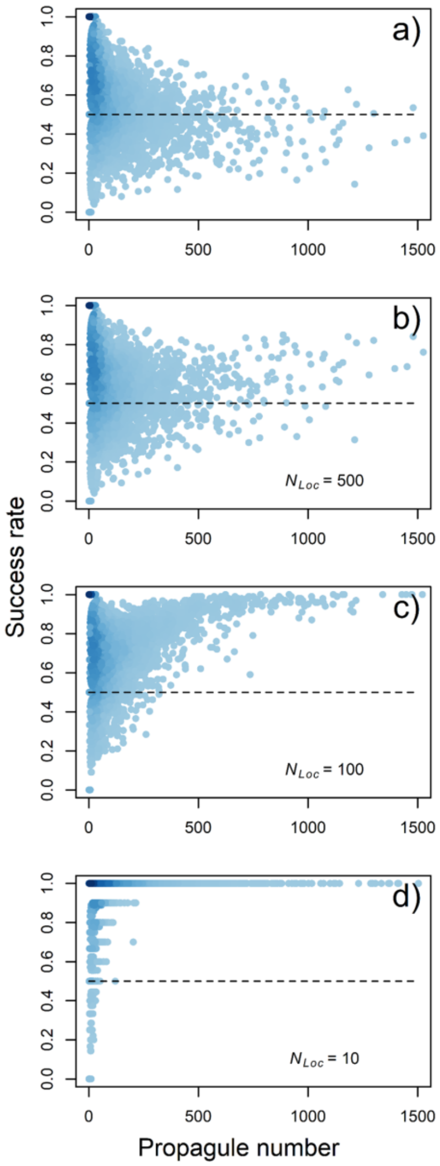

**Figure S1**. Relative support (AIC weight, AICW) for species level models (see Materials and Methods) including the effects of propagule pressure (blue), species trait λ (red) or both propagule pressure and λ (green), and for different strengths of correlation between propagule number and the trait λ. Results shown the mean AICW across *n* = 100 simulations.