REVIEW ARTICLE

Plant secondary metabolites and primate food choices: A meta-analysis and future directions

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Abstract

The role of plant secondary metabolites (PSMs) in shaping the feeding decisions, habitat suitability, and reproductive success of herbivorous mammals has been a major theme in ecology for decades. Although primatologists were among the first to test these ideas, studies of PSMs in the feeding ecology of non-human primates have lagged in recent years, leading to a recent call for primatologists to reconnect with phytochemists to advance our understanding of the primate nutrition. To further this case, we present a formal meta-analysis of diet choice in response to PSMs based on field studies on wild primates. Our analysis of 155 measurements of primate feeding response to PSMs is drawn from 53 studies across 43 primate species which focussed primarily on the effect of three classes of PSMs tannins, phenolics, and alkaloids. We found a small but significant effect of PSMs on the diet choice of wild primates, which was largely driven by the finding that colobine primates showed a moderate aversion to condensed tannins. Conversely, there was no evidence that PSMs had a significant deterrent effect on food choices of non-colobine primates when all were combined into a single group. Furthermore, within the colobine primates, no other PSMs influenced feeding choices and we found no evidence that foregut anatomy significantly affected food choice with respect to PSMs. We suggest that methodological improvements related to experimental approaches and the adoption of new techniques including metabolomics are needed to advance our understanding of primate diet choice.

KEYWORDS
meta-analysis, metabolomics, phylogeny, plant secondary metabolites, tannins

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1 | INTRODUCTION

The role that plant secondary metabolites (PSMs) play in shaping the feeding decisions, habitat choices, and reproductive success of herbivorous mammals has been a major theme in ecology since Freeland & Janzen (1974) first laid out a research framework and a series of hypotheses almost 50 years ago. Although primatologists were among the first to test these predictions (Glander, 1978, 1982; Hladik, 1977; Milton, 1979; Oates et al., 1977; Wrangham & Waterman, 1981), studies of the role of PSMs in primate feeding ecology have lagged in recent years. There have been a handful of studies on phytochemicals (Lu et al., 2011; Wasserman et al., 2012, 2013) and particular bioactive compounds identified or presumed in medicinal plant use by primates (Huffman, 1997; Krief et al., 2006). Nevertheless, nonhuman primates have not featured in some of the recent advances in the field, including the impact of PSMs on reproductive success (DeGabriel et al., 2009; McArt et al., 2009), the mechanisms by which animals regulate their intake of PSMs (Torregrossa & Dearing, 2009), and the role of the microbiome in detoxification of ingested PSMs (e.g., Kohl et al., 2014). This is a missed opportunity given the extraordinary long-term data that have been collected on the daily food choices of individual primates. No other plant−mammal system matches the extent and depth of knowledge of the food choices of wild primates.

Other plant−mammal systems have the advantage of captive studies that allow bioassays and thus facilitate the identification of PSMs that are important influences on diet choice and nutrition (Bryant et al., 1983; Pass et al., 1998). Within these nonprimate plant−mammal systems, the effects of PSMs on feeding are stronger in captive studies (where concentrations of isolated PSMs can be controlled and measured; DeGabriel et al., 2014), than in studies investigating the diet of free-ranging herbivores. This can give the impression that food choices are controlled by a few simple factors. In natural environments; however, wild animals have many more choices and not surprisingly, the factors affecting diet selection are naturally more complex and difficult to identify.

Variations in digestive physiology among primate species have long been speculated as a major difference in the capacity of primates to tolerate dietary PSMs. In particular, foregut-fermenting colobines are thought to be at an advantage over hindgut-fermenting non-colobine species in consuming diets rich in secondary metabolites due to the additional opportunities for detoxification in the forestomach (Freeland & Janzen, 1974; Kay & Davies, 1994; Mowry et al., 1996). Foregut fermenters can be divided into two groups: those species with a tripartite stomach and those having a praesaccus, or quadripartite stomach (e.g., species of the genera *Procolobus*, *Piliocolobus*, *Rhinopithecus*, *Pygathrix*, and *Nasalis*) (Hoshino et al., 2021; Langer, 1988).

Although much has been done to use field-based feeding observations to understand the nutrient targets of primates (Felton et al., 2009; Norconk & Conklin-Brittain, 2004; Raubenheimer et al., 2015; Rothman et al., 2011; Takahashi et al., 2019; Uwimbabazi et al., 2021; Wrangham et al., 1998), there is a striking absence of data

on the effect that specific PSMs might play in either influencing food choice or modifying nutrient targets. In contrast to studies with marsupials (Marsh et al., 2015), rodents (Sorensen et al., 2005), lagomorphs (Bryant et al., 1983), and ungulates (Stolter et al., 2005), few studies on primates have focussed on specific, characterized secondary metabolites. Rather, the majority of studies have relied on broad compound categories such as "total phenolics," "condensed tannins," and "alkaloids." The problems associated with relying on these broad categories to characterize plant chemistry particular of large trees in diverse environments have been well-documented (Rautio et al., 2007; Rothman, Dusinberre, et al., 2009; Salminen & Karonen, 2011; Waterman & Mole, 1994). However, researchers persist because these assays are readily accessible and alternatives are not necessarily simple to use, require advanced analytical instrumentation, and often require specific technical expertise to operate.

Stalenberg et al. (2022) argued that going beyond crude categories of PSMs and identifying specific compounds important in the feeding ecology of primates is an important step in linking nutrition to population dynamics. In support of these arguments, we present here a formal meta-analysis of the role of plant secondary compounds in primate diet selection based on data pulled from published literature. By identifying and partitioning the variance in the true effect of PSMs on primate diet selection due to individual species and their phylogenetic relationships, digestive physiology (e.g., foregut-fermenting colobine monkeys vs. hindgut-fermenting non-colobines), categories of secondary metabolites, and methodological approaches, we summarize the current state of knowledge in this area of primate nutritional ecology and make suggestions for future progress.
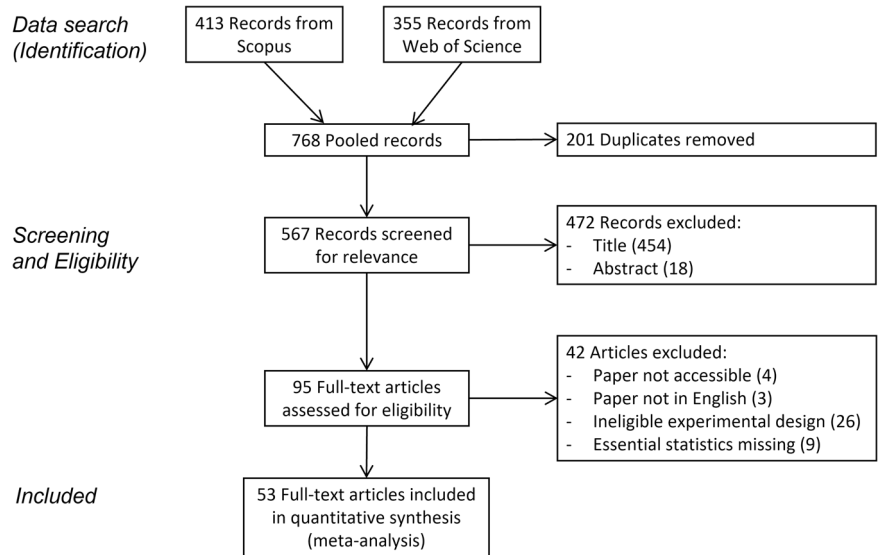
2 | MATERIALS AND METHODS

2.1 | Literature search

We searched published literature for studies that reported the effect of a PSM on diet choice or diet choices by a wild primate. Criteria for inclusion were broad. We included studies that measured any PSM, primate species, plant species, plant part, and any method for determining diet choice. The literature search (last updated on January 10, 2022) was performed using both *ISI Web of Science* and *Scopus* as these can produce different results. To capture all relevant papers, we used the following keywords, without limitations on search categories; (primat* OR monkey* OR lemur* OR prosim* OR apes) AND (feed* OR food* OR diet* OR nutrition*) AND ("secondary metab*" OR "secondary compoun*" OR "plant metab*" OR "plant compoun*" OR "plant chem*" OR phenolic* OR tannin* OR terpene* OR alkaloid* OR cyan*).

Our search yielded 567 unique citations (355 items from *Web of Science* and 413 items from *Scopus* with 201 duplicates, Figure 1). These were initially assessed based on title and abstract, reducing the list to 95 unique references. The studies to be included in the final data set were observation-based field studies on the diet of primates

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FIGURE 1 Summary of paper selection process. A reference list for all papers selected for inclusion in the final data set can be found in Supporting Information: Table S1.

Data search (Identification)

413 Records from Scopus

355 Records from Web of Science

768 Pooled records → 201 Duplicates removed

Screening and Eligibility

567 Records screened for relevance → 472 Records excluded:
- Title (454)
- Abstract (18)

95 Full-text articles assessed for eligibility → 42 Articles excluded:
- Paper not accessible (4)
- Paper not in English (3)
- Ineligible experimental design (26)
- Essential statistics missing (9)

Included

53 Full-text articles included in quantitative synthesis (meta-analysis)

and were limited to those that were published in English. Studies conducted in zoos or with manipulated diets were excluded. Studies had to contain suitable data regarding the concentration, or presence/absence of PSMs, and some measure of choice or preference or selection exhibited by the focal primate species. Finally, studies that failed to provide sufficient details to enable the calculation of effect size were excluded ($n = 9$, Figure 1). After conforming to these parameters, 53 studies remained that broadly fell into two categories: those that compared the PSM concentrations in foods eaten compared with plant parts that were not eaten, and studies that examined choices of primates for foods along a gradient of PSM concentration, or prevalence. Studies generally measured either condensed tannin, hydrolyzable tannin, total tannin, or total phenolics, and often combinations of these were measured for the same food item. Fewer studies measured the effect of alkaloids (7) and saponins (2) on food choice and there were no studies suitable for inclusion in the final data set that measured terpene concentration or specific compounds within these broad chemical classes.

2.2 | Effect size calculations

Effect sizes were calculated from each included study to provide a consistent measure of the effect of PSMs on diet choice in primates across multiple studies. This approach is becoming increasingly common within ecology, as it offers many advantages over qualitative reviews. It is particularly powerful in facilitating quantitative analysis of effects across multiple studies, species, and systems while providing a consistent approach to weighting individual results with respect to their variance (for reviews, see Borenstein et al., 2009; Koricheva et al., 2013; Nakagawa & Cuthill, 2007). This offers advantages over narrative-based reviews or "vote-counting" approaches which do not offer a transparent or

repeatable method for considering variable quality within and among studies. Bias-corrected standardized mean difference (Hedges' g) was the effect size calculated from each study. Order of preference for data used to calculate Hedges' g was (1) means and standard deviations, or log odds ratios from raw data, (2) test statistics (e.g., Mann−Whitney U statistics, correlation coefficients, t statistics), (3) means and standard deviations extracted from figures, and finally, (4) medians and interquartile ranges (IQR). Some papers presented sufficient data for multiple effect sizes to be calculated, as often multiple PSMs were studied in each paper, and/or multiple species of primate were examined. When a pooled sample size was presented, it was assumed that the two treatment groups had equal sample sizes. Where median and interquartile ranges were given, the mean was assumed to be equal to the median, and the standard deviation was equal to IQR/1.35. In calculating log odds ratios, when the count of samples containing a PSM was zero, an effect size was not calculated and was excluded, as no variance in PSM concentration can be calculated where $n = 0$. Where effect sizes were readily calculated in other forms (e.g., correlation coefficients, or log odds ratios), these were converted to Hedges' g (Borenstein et al., 2009; Koricheva et al., 2013). Studies were checked to ensure the direction of the effect was correctly interpreted; particularly where nonparametric statistical tests were conducted. The variance of Hedges' g was also calculated using the formulas of Borenstein et al. (2009).

For example, Ganzhorn et al. (1985) presented data on the condensed tannin concentrations in leaves of trees selected by Woolly indri (*Avahi laniger*), and condensed tannin concentrations of leaves of trees that were not chosen. From these data, the mean and standard deviation and sample size of condensed tannin concentration of leaves from trees observed to being eaten (mean CT: 47.32, SD: 24.44, $n = 12$) and leaves from trees not observed to be eaten (mean CT: 28, SD: 21.6, $n = 18$) were calculated. This computes a Hedge's g of 0.83 and variance of 0.14. The positive direction of the effect size indicates a preference

for higher condensed tannin concentrations. Norscia et al. (2006) examined the food preference of Verreaux's sifaka (*Propithecus verreauxi*). A Spearman rank correlation between condensed tannin concentration of leaves and time spent feeding on that particular species was presented (Spearman $r = -0.61$, $n = 28$). This computes a Hedge's g of -1.57 and variance of 0.24. The negative direction of the effect size indicates a preference for tree species with lower concentrations of condensed tannin.

Finally, where multiple comparisons within a single study shared the same control, a composite Hedges' g and variance were calculated for use in standard random-effects meta-analysis models. We took the conservative approach and assumed a complete within-study correlation between effects that shared a control group ($r = 1$) when computing the composite variance (Borenstein et al., 2009; Ganzhorn et al., 2017). The same approach was taken in computing a single, composite effect size for each study. An alternative approach was taken for multilevel mixed-effects meta-analysis, where a within-study level random factor was included to account for nonindependence between observations, within studies. Formulae for calculating effect sizes are detailed in Borenstein et al. (2009) and Koricheva et al. (2013). Effect sizes were calculated in R (version 4.0.3) with the R package "compute. es" (version 0.2-5., Del Re, 2013).

2.3 | Differences among primate groups

We divided the studies into two groups: those that consider the subfamily Colobinae ("colobines") and those that consider the other 10 families and subfamilies ("non-colobines") to investigate the role of digestive anatomy (foregut and hindgut) in influencing the response of primates to PSMs.

In Section 4 below, we urge caution in how these analyses are used given the unevenness of the available data and the large diversity encompassed by the non-colobine grouping.

2.4 | Moderator variables for meta-analysis

Inverse-weight random-effects and mixed-effects (meta-regression) meta-analyses were used to determine if primates exhibited diet choice in relation to PSMs. Primate group and PSM type could explain variation in effect sizes. Primates were classified into two groups to differentiate between the colobine monkeys (COL) due to the difference in their digestive physiology from all other primates (OTH). PSMs were grouped into six groups: condensed tannins (CT), hydrolyzable tannins (HT), alkaloids (AL), saponins (SA), and total phenols (TP). Where the radial diffusion method (Hagerman, 1987) had been used to measure condensed tannins, these were categorized as total tannins (TT). We included a two-level factor on experimental methodology: studies that compared eaten foods to noneaten

foods (NE) and those that examined food preference along a PSM gradient (VP).

2.5 | Random-effects meta-analyses

Standard random-effects meta-analysis models (function "rma") were fitted to the data, using the R package "metafor" (version 3.0-2, Viechtbauer, 2010). To address issues of nonindependence between effect sizes within studies, composite effect sizes for each study were computed (as described above) to produce a single effect size per study. In testing for the effect of the primate group, a single effect size for each primate group was computed for each study, if both primate groups were represented within the same study. In testing for the effect of PSM on choice, a single composite effect for each PSM type per study was computed. A random-effects meta-analysis was fitted to either a composite data set of a single effect size per study to generate an overall effect of PSMs on primates or a data set of composite effect sizes by PSM type. Random-effects meta-analysis models were fitted to test for (a) an overall effect of PSMs on primate food choice followed by analyses to examine the effects of PSMs on primate diet choice by (b) primate group, (c) PSM type, and (d) PSM type within each primate group. A random-effects meta-analysis was performed on the experimental method (e), in which studies were categorized into one of two groups based on their methodology: those that compared eaten foods to noneaten foods (NE), and those that examined food choice along a PSM gradient (VP). These analyses were undertaken by filtering the data set to each category and fitting a random-effects meta-analysis model to the filtered data. As there was only a single study that measured foliar saponins, these effect sizes ($n = 2$) were excluded from the meta-analysis. Likewise, only a single effect size was present for a colobine monkey and an alkaloid, hydrolyzable tannin, and only two effect sizes for total tannin, so these were not analyzed in the standard random-effects analyses explicitly and were removed entirely from the multilevel meta-analysis. Tests for model robustness were performed with Rosenberg's failsafe N. Tests for publication bias were performed with Egger's regression test on funnel plot asymmetry, and trim and fill analyses in the R package "metafor".

2.6 | Multilevel (phylogenetic) random-effects meta-analyses

Multilevel random-effects meta-analyses provide a means of quantifying heterogeneity among effects, including those attributable to individual studies (including multiple measures from within a study), between-study effects, factors specific to species, and relatedness among species (Borenstein et al., 2009; Cinar et al., 2022; Nakagawa & Santos, 2012). Phylogenetic relatedness can be an important source of nonindependence within ecological meta-analyses (Chamberlain

et al., 2012; Cinar et al., 2022). To account for phylogenetic relatedness among species, a phylogenetic variance−covariance matrix was derived from a phylogeny of primates (Arnold et al., 2010; Bray et al., 2018). Branch lengths were extracted for those species that were present in the meta-analysis data set, and the subtree scaled to a root height of 1 and raised to the power of 1. Not all primates in the effect size data set are present in the phylogeny, so this was dealt with in three ways. First, where species nomenclature has been revised since studies were published, species names were updated to reflect the taxonomy of Arnold et al. (2010). Second, where missing species had sister taxa in the phylogeny (e.g., same genus), the missing species were included as a node alongside their sister taxa. This was done for two species. Finally, where a species did not have sister taxa present, they were not included in the analyses ($n = 1$). The variance−covariance matrix was calculated from the branch lengths and included in the meta-analysis model as a random effect, using the R package "ape" (Paradis & Schliep, 2019). A within-study ID was included to account for nonindependence of effect sizes from within studies. This approach allows for studies to contribute multiple effects to the multilevel random-effects meta-analysis. Within-study ID and study were included along with species and phylogenetic relatedness as random factors. Heterogeneity among effects attributable to the random effects, including the total proportion of variation classifiable as heterogeneity (I^2) and Cochran's Q statistic were calculated along with the estimated effect size ±95% confidence intervals. In addition to a null model without moderator variables (i.e., a random-effects meta-analysis), primate group, PSM type, and the interaction between PSM type and primate group were included in successive mixed-effects meta-analysis models as moderator variables. A further analysis was undertaken with experimental type included as a moderator variable. Tests for model robustness and publication bias were conducted using fail-safe N, Eggers regression test on model residuals, and trim-and-fill analysis on model residuals of the multilevel random-effects meta-analysis model (e.g., without moderator variables). Adjustment to the mean Hedges' g estimate of the multilevel random-effects model was made as per Sutton et al. (2011) to evaluate the effect of publication bias on the mean effect. Figures were produced in R with "ggplot2" and "ggtree" packages (Yu et al., 2017).

2.7 | Effects of variation in foregut anatomy among colobine primates

Preliminary analyses suggested an effect of condensed tannins on diet choice in colobine primates. Given this result and the availability of sufficient effect sizes, we further explored the role of foregut anatomy in this group by filtering the data set used for the multilevel analysis to just the colobine primates. We separated the colobine primates into two categories based on foregut anatomy: those species with a tripartite stomach (TRI) were distinguished from those having a praesaccus (e.g., species of the genera *Rhinopithecus*, *Procolobus*, and *Piliocolobus*), as part of a quadripartite stomach (QUAD) (Langer, 1988). As there were only

11 species, an analysis of the composite data set was not produced; hence only a multilevel meta-analysis was conducted. Colobine primate phylogeny was extracted from the phylogeny described above and a variance−covariance matrix was calculated for inclusion as a random factor. The additional effect sizes on alkaloids, saponins, and total tannins were retained for the analyses including stomach anatomy as the moderator variable. However, this was restricted to condensed tannins and total phenolics to examine the interaction between stomach anatomy and PSM type, due to there being insufficient effect sizes of the other PSM compounds to produce a reliable estimate. Model robustness and publication bias were examined as described above; however, a trim-and-fill analysis was not performed, as a random effects (null) model was not produced.

3 | RESULTS

A total of 155 effect sizes were drawn from 53 studies, covering 43 species of primates. For the random effects meta-analysis (in which each study contributes a single effect size estimate), 53 effect sizes were available for testing the overall effect of PSMs on primate diet choice. Seven individual effect size estimates from the literature were exceptionally large (Hedges' $g < -3.0$). These large effect sizes were associated with relatively small sample sizes and relatively large standard errors. These large effect sizes were excluded from the multilevel (phylogenetic) mixed-effects meta-analysis presented here. Once the data set was restricted to match the species available in the phylogenetic tree (1 species, 3 effect sizes removed), and removing the effects sizes for saponin ($n = 2$), alkaloids ($n = 1$), hydrolyzable tannins ($n = 1$) and total tannins ($n = 2$), and outlier effect sizes (Hedges' $g < -3.0$, studies = 4, species = 2, effect sizes = 7), 139 effect sizes from 48 studies representing 40 species of primate were available for the multilevel phylogenetic random-effects meta-analyses. When restricted further to just the colobine primates, a total of 41 effect sizes across all PSMs were available for analysis, representing 11 species of colobine primates. By performing both standard random-effects and multilevel (phylogenetic) meta-analysis, we present a nonconservative (random-effect meta-analysis) and conservative (multilevel [phylogenetic] random-effects meta-analysis) models to convey the sources of variation in effect sizes. Hereafter, mean effect sizes are presented as the mean Hedges' g, and 95% confidence interval as mean Hedges' g [95% CI].

3.1 | Random-effects meta-analysis models

A standard random-effects meta-analysis revealed a significant negative effect of PSMs on primate feeding choice (mean effect size $g = -0.31$ [−0.520 to −0.081]; Table 1, Figure 2). The variance of the estimated true effect is relatively large ($Tau^2 = 0.487$), indicating a moderate degree of between-study heterogeneity. Likewise, the I^2 statistic is large

($I^2 = 84.14\%$), suggesting that a considerable proportion of the between-study variation is real and thus, potentially explainable.

A random-effect meta-analysis model to test for differences between colobine and non-colobine primates revealed that PSMs negatively affected feeding in the colobines (mean Hedges' $g = -0.209$ [-0.444 -0.027], Table 1). Likewise, there was no significant effect of PSMs on feeding by non-colobine primates (mean Hedges' $g = -0.174$ [-0.357 to 0.008], Table 1). Filtering to PSM type showed that condensed tannins had a significant effect on reducing food preferences (mean Hedges' $g = -0.402$ [-0.696 to -0.108], Table 1). In contrast, there was no significant effect of alkaloids, hydrolyzable tannins, total phenolics, or total tannins on the diet choice of primates (Table 1).

We explored this further by testing whether colobine and non-colobine primates differed in their response to condensed tannin. This analysis showed that the effect of condensed tannins as a deterrent in diet selection was restricted to the colobine primates (mean Hedges' $g = -0.520$ [-0.900 to -0.139], Table 1). No other PSM was found to have a significant effect on colobine monkey diet selection. Likewise, no PSM was found to have a significant effect on the diet selection of non-colobine primates (Table 1, Figure 2).

In terms of the potential role of experiment design, there was a significant effect of PSMs on primate diet selection in studies that compared eaten to not eaten foods (mean Hedges' $g = -0.389$ [-0.718 to -0.060], Table 1), while those studies that examined diet choice by examining variable preference between eaten foods with differing concentrations of PSMs did not detect a significant effect of PSMs on diet selection (Table 1).

3.2 | Heterogeneity and publication bias in standard random-effects meta-analysis

The robustness of the standard random-effects meta-analysis models was generally poor, with Rosenberg's "failsafe N" being considerably smaller than the threshold value of $>5k+10$ (where k is the number of effects) for all models (Table 2). Egger's regression test on Pearson residuals from the fitted models revealed that significant funnel plot asymmetry was present in some of the models, including the overall test of effects of condensed tannins on primates (Egger's $t = -3.11$, $p = 0.003$, Table 2, Supporting Information: Figure S1). However, many of these are likely being influenced by the few, large effect sizes within the data set (Supporting Information: Figure S1). For example, there was significant asymmetry in the non-colobine group in relation to condensed tannins (non-colobine: CT, Table 2). A trim and fill analysis on the random-effects meta-analyses indicated that there may be missing effects on the right side of the funnel plot for the model on total phenolics (missing effects = 1); however, the inclusion of the missing value had no significant effect (mean Hedges' $g = -0.004$, $p = 0.961$; Table 2).

TABLE 1 Summary statistics of standard random effects meta-analysis models, with large effect sizes included.

Coefficient	k	Betas	ci. lower	ci. upper	zval	pval	Tau^2	Q	dfQ	pvalQ	I^2
All	53	−0.301	−0.520	−0.081	−2.687	**0.007**	0.487	201.635	52	**<0.001**	84.141
Colobine	19	−0.209	−0.444	0.027	−1.739	0.082	0.105	32.796	18	**0.018**	41.037
Non-colobine	69	−0.174	−0.357	0.008	−1.878	0.060	0.404	225.863	68	**<0.001**	75.284
Alkaloids	6	−0.223	−0.644	0.198	−1.038	0.299	0.107	7.647	5	0.177	41.213
Condensed T	43	−0.402	−0.696	−0.108	−2.680	**0.007**	0.750	176.361	42	**<0.001**	84.198
Hydrolyzable T	10	0.061	−0.198	0.321	0.464	0.643	0.055	15.467	9	0.079	32.701
Total phenolics	24	−0.019	−0.183	0.144	−0.230	0.818	0.023	41.415	23	**0.011**	14.278
Total T	5	0.091	−0.537	0.720	0.285	0.776	0.312	10.614	4	**0.031**	61.871
Non-colobine: AL	6	−0.223	−0.644	0.198	−1.038	0.299	0.107	7.647	5	0.177	41.213
Non-colobine: CT	33	−0.376	−0.758	0.006	−1.930	0.054	1.023	152.490	32	**<0.001**	88.237
Non-colobine: HT	9	0.023	−0.266	0.312	0.156	0.876	0.065	14.193	8	0.077	34.892
Non-colobine: TP	17	−0.058	−0.317	0.202	−0.437	0.662	0.131	38.923	16	**0.001**	49.047
Non-colobine: TT	4	−0.009	−0.749	0.730	−0.025	0.980	0.383	9.292	3	**0.026**	68.243
Colobine: CT	10	−0.520	−0.900	−0.139	−2.675	**0.007**	0.189	19.473	9	**0.021**	53.329
Colobine: TP	7	−0.019	−0.302	0.264	−0.132	0.895	0.000	2.488	6	0.870	0.000
Not eaten	33	−0.389	−0.718	−0.060	−2.320	**0.020**	0.766	153.815	32	**<0.001**	91.292
Variable pref	20	−0.184	−0.474	0.107	−1.239	0.215	0.236	47.817	19	**<0.001**	58.870

Note: Bold values denote $p < 0.05$.

Abbreviations: AL, alkaloids; CT, condensed tannins; HT, hydrolysable tannins; T, tannin; TP, total phenolics; TT, total tannin.

FIGURE 2 Forest plot of standard random effects and multilevel (phylogenetic) mixed effects meta-analyses. Standard random effects meta-analyses include the large effect sizes (Hedges' $g < -3.0$), while the multilevel models had the large effect sizes omitted. Mean Hedges' $g \pm 95\%$ CI are presented. AL, alkaloids; CT, condensed tannins; HT, hydrolysable tannins; T, tannin; TP, total phenolics; TT, total tannin.
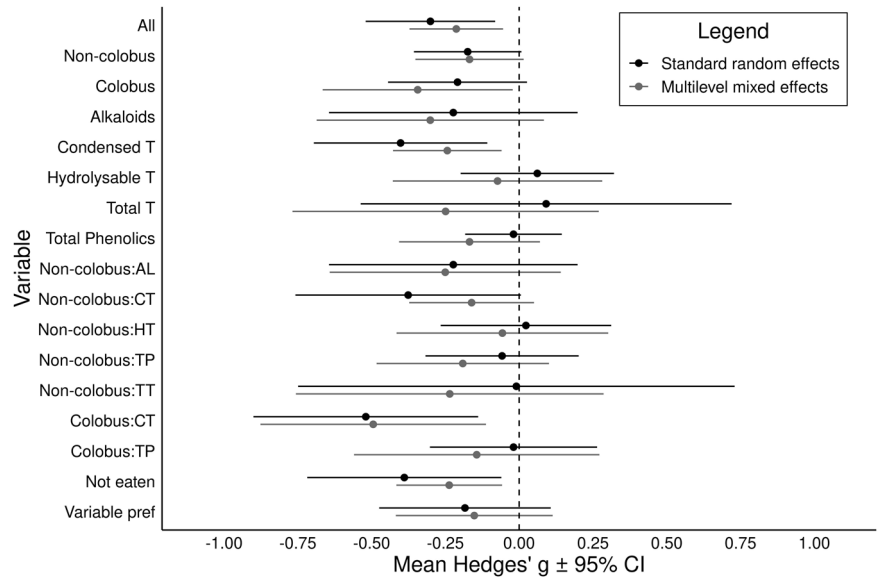

TABLE 2 Summary of fail-safe N, Egger's regression test, and trim-and-fill tests for robustness and publication bias in standard random effects meta-analysis models.

Coefficient	Failsafe N	Eggers zval	Eggers df	Eggers pval	TAF, missing k	t_beta	t_se	t_ci. lb	t_ci. ub	t_pval
All	60	−3.047	51	**0.004**	0					
Colobine	0	−1.568	17	0.135	0					
Non-colobine	14	−2.397	67	**0.019**	0					
Alkaloids	0	0.345	4	0.747	0					
Condensed T	90	−3.110	41	**0.003**	0					
Hydrolyzable T	0	1.104	8	0.302	0					
Total phenolics	0	−1.997	22	0.058	1	−0.004	0.086	−0.173	0.164	0.961
Total T	0	0.868	3	0.449	0					
Colobine: CT	21	−1.737	8	0.121	0					
Colobine: TP	0	0.076	5	0.942	0					
Non-colobine: AL	0	0.345	4	0.747	0					
Non-colobine: CT	6	−2.514	31	**0.017**	0					
Non-colobine: HT	0	1.434	7	0.195	0					
Non-colobine: TP	0	−1.972	15	**0.067**	0					
Non-colobine: TT	0	0.502	2	0.665	0					
Not eaten	22	−2.894	31	**0.007**	0					
Variable pref	0	−1.524	18	0.145	0					

Note: Abbreviations as per Table 1. Bold values denote $p < 0.05$.

3.3 | Multilevel phylogenetic random-effects meta-analysis models

Fitting a multilevel (phylogenetic) random-effects meta-analysis with additional random effects to capture nonindependence among studies, within studies, primate species, and phylogenetic relationships among species indicated a significant effect of PSMs on primate feeding choices (mean Hedges' $g = -0.213$ [−0.371 to −0.055]; Table 3). Variation was largely captured by residual (within-study) effects (I^2 Residual = 26.80%) and Species (I^2 Species = 27.95%). Significant heterogeneity among effects remained ($Q_{139} = 380.45$, $p < 0.001$), suggesting that additional factors may still explain the variance among effects.

TABLE 3 Summary multilevel (phylogenetic) mixed-effects meta-analysis models with large effect sizes omitted.

Coefficient	k	Betas	ci. lower	ci. upper	zval	pval	Q	dfQ	pvalQ	QM	dfQM	pvalQM	I^2 Residual	I^2 Study	I^2 Species	I^2 Phylogeny	I^2 Total
All	139	−0.213	−0.371	−0.055	−2.635	**0.008**	380.453	138	**<0.001**				26.799	7.347	27.955	<0.001	62.101
Non-colobus	139	−0.168	−0.351	0.015	−1.798	0.072											
Colobus	139	−0.344	−0.666	−0.022	−2.093	**0.036**	380.238	138	**<0.001**	7.613	1	**0.022**	26.658	8.706	26.897	<0.001	62.261
Alkaloids	139	−0.301	−0.686	0.084	−1.533	0.125											
Condensed T	139	−0.243	−0.427	−0.060	−2.596	**0.009**											
Hydrolyzable T	139	−0.073	−0.428	0.281	−0.405	0.685											
Total Phenolics	139	−0.168	−0.407	0.070	−1.381	0.167											
Total T	139	−0.249	−0.768	0.270	−0.942	0.346	323.040	138	**<0.001**	8.549	4	0.128	30.668	4.077	26.189		60.933
Non-colobus: AL	139	−0.250	−0.642	0.141	−1.254	0.210											
Non-colobus: CT	139	−0.161	−0.372	0.050	−1.493	0.135											
Non-colobus: HT	139	−0.057	−0.415	0.302	−0.311	0.756											
Non-colobus: TP	139	−0.191	−0.483	0.101	−1.282	0.200											
Non-colobus: TT	139	−0.235	−0.756	0.286	−0.884	0.376											
Colobus: CT	139	−0.495	−0.877	−0.112	−2.537	**0.011**											
Colobus: TP	139	−0.144	−0.560	0.272	−0.678	0.498	316.115	138	**<0.001**	10.546	6	0.160	29.735	6.582	24.612	<0.001	60.928
Not eaten	139	−0.237	−0.416	−0.058	−2.593	**0.010**											
Variable pref	139	−0.152	−0.418	0.114	−1.122	0.262	375.401	138	**<0.001**	7.280	1	0.026	27.387	6.977	27.758	<0.001	62.123

Note: Abbreviations as per Table 1. Bold values denote *p* < 0.05.

A multilevel mixed-effects meta-regression model with primate group as moderator variable revealed that colobine monkey diet selection is negatively impacted by PSMs (mean Hedges' $g = -0.344$ [−0.666 to −0.022], Table 3, Figure 2). Conversely, non-colobine primates did not exhibit any significant response to PSMs in their diets (mean Hedges' $g = -0.168$ [−0.351 to 0.015], Table 3, Figure 2). The moderator variable was significant ($QM_1 = 7.61$, $p = 0.022$). Heterogeneity among effects was largely attributable to residual effects (I^2 Residual = 26.65%) and species (I^2 Species = 26.90%, Table 3). Further exploration at the species level revealed that all colobine primate species exhibited variably significant yet consistent, negative responses to PSMs (Figure 3). Conversely, considerable variation in responses in non-colobine primates was apparent, including significant positive, nonsignificant, and significant negative responses that do not appear related to phylogeny (Figure 3).

Inclusion of the interaction between primate group and PSM type as a moderator variable in a multilevel mixed-effects meta-regression model revealed that colobine monkeys show a significant aversion in diet selection to condensed tannins with a mean Hedges' g of −0.495 [−0.877 to −0.112], which is considered a moderate-sized effect (Borenstein et al., 2009; Table 3, Figure 2). Colobine monkeys did not show any significant response to any other PSM compound, while non-colobine primates showed no significant response to any of the PSM compounds (Table 3). The moderator variable, however, was nonsignificant ($QM_6 = 10.54$, $p = 0.16$).

The inclusion of study experiment type (eaten/non-eaten vs. variable preference for food) in a multilevel mixed-effects meta-regression model revealed that there was a significant effect of study methodology on whether PSMs were found to affect diet selection in primates (Table 3). Studies comparing PSMs between foods that were eaten and nonfood plant parts found a significant effect of PSMs on primate food choice (mean Hedges' $g = -0.237$ [−0.416 to −0.058], Table 3, Figure 2). Conversely, studies exploring diet choice in relation to PSMs along a gradient of primate food choices did not find a significant effect of PSMs on primate diet choice (mean Hedges' $g = -0.152$ [−0.418 to 0.114], Table 3, Figure 2).

Finally, restricting the analysis to colobine primates separated into two groups based on digestive physiology found no evidence that digestive physiology significantly influenced the role of PSMs in diet choice in colobine primates. The multilevel analysis indicated that the tripartite colobine primates exhibit a nonsignificant response to PSMs in general (mean Hedges' $g = -0.36$ [−0.81 to 0.091]); however, they show a significant aversion to condensed tannins (mean Hedges' $g = -0.421$ [−0.809 to −0.033], Table 4 and Supporting Information: Figure S2). Quadripartite primates exhibited an identical response, showing a nonsignificant response to all PSMs, but a significant negative response to condensed tannins (Table 4 and Supporting Information: Figure S2). Furthermore, there is no evidence to suggest that there are different responses between tripartite and quadripartite colobine primates (Table 4 and Supporting Information: Figure S2). The moderator variable of colobine foregut type and
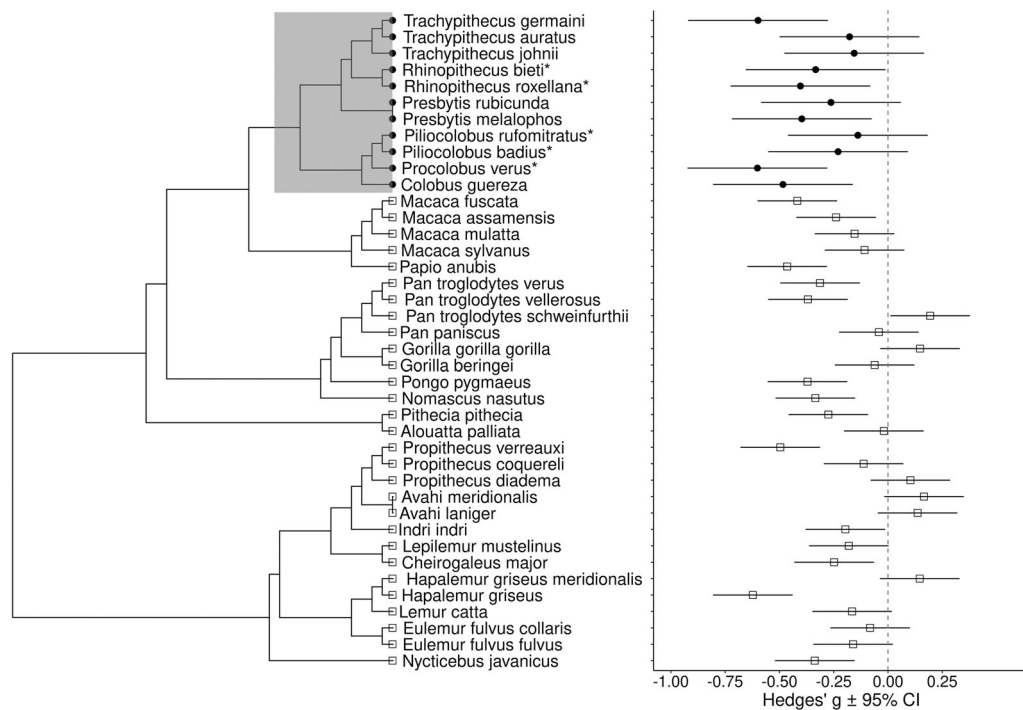

FIGURE 3 Predicted individual species-level response (mean Hedges' g ± 95% CI) to plant secondary metabolites derived from the multilevel (phylogenetic) mixed effect model, with primate group as moderator variable. Individual species-level response includes the fixed effect (primate group) + random effect of Species and Phylogeny. The primate phylogeny is presented on the left-hand side, with the colobine primates shaded in gray. Colobine primates with quadripartite stomachs are denoted with an asterisk.

TABLE 4 Summary of multilevel (phylogenetic) meta-regression models on colobine primate response to PSMs with respect to foregut anatomy (Tri or Quad) and PSM type (CT, TP).

Coefficient	k	Betas	ci.lower	ci.upper	zval	pval	Q	dfQ	pvalQ	QM	dfQM	pvalQM	I^2 Species	I^2 Phylogeny	I^2 Study	I^2 Residual	I^2 Total
Quad		−0.309	−0.760	0.141	−1.346	0.178											
Tri	41	−0.360	−0.810	0.091	−1.565	0.118	47.746	40	0.159	4.262	1	0.119	<0.001	<0.001	49.243	<0.001	49.243
Quad:CT		−0.493	−0.984	−0.003	−1.970	**0.049**											
Tri:CT		−0.421	−0.809	−0.033	−2.124	**0.034**											
Quad:TP		−0.142	−0.602	0.318	−0.605	0.545											
Tri:TP	41	−0.190	−0.641	0.260	−0.829	0.407	36.948	40	0.291	10.573	7	0.227	29.643	<0.001	<0.001	<0.001	29.643

Abbreviations: CT, condensed tannins; PSM, plant secondary metabolites; Quad, quadripartite; TP, total phenolics; Tri, tripartite. Bold values denote $p < 0.05$.

the interaction between foregut type and PSM type was nonsignificant (Table 4).

3.4 | Heterogeneity and publication bias in multilevel phylogenetic random-effects meta-analysis

I^2 statistics revealed that residual (within-study) effects were found to explain much of the explainable variation in effects (Table 3). Likewise, primate species were a major factor in explaining variation among effects (Table 3). Conversely, phylogeny and study-level explained a relatively small proportion of heterogeneity between effects (Table 3). Total heterogeneity ranged between 60.93% and 62.26%, suggesting a moderate degree of heterogeneity is not associated with purely measurement error; however, the moderator variables explored did not explain a substantial proportion of this heterogeneity (Table 3). Heterogeneity was markedly less in the analysis restricted to colobine primates, with total heterogeneity being 49.24% and 29.64% for the analysis on colobine stomach type and the interaction between stomach type and PSM type, respectively (Table 4).

A test for the "file drawer effect" was conducted on the multilevel (phylogenetic) random-effects meta-analysis model suggested that model robustness was good, with a fail-safe N of 1178. This is greater than the $5k + 10$ value of 705 used as a threshold for determining model robustness to unpublished studies. Egger's regression test on the meta-analysis model residuals indicated no significant bias ($z = -1.14$, $p = 0.253$). A trim-and-fill analysis on residuals indicated 27 missing effect sizes, and a significant effect on the mean of residuals (mean Hedges' $g = 0.404$, $p < 0.001$, Supporting Information: Figure S3). Adjusting mean Hedges' g, the multilevel random-effects model to account for the residual bias resulted in the overall effect becoming positively significant, suggesting that there may be a real effect of missing studies (bias-corrected mean Hedges' $g = 0.191$ [0.032 to 0.349]).

Egger's regression tests on analyses restricted to colobine primates did not detect significant bias in model residuals with respect to PSMs (Eggers $z = -1.67$, $p = 0.094$) or the interaction between PSM type and dietary physiology (Eggers $z = -1.75$, $p = 0.079$).

4 | DISCUSSION

4.1 | Do PSMs affect feeding by primates?

While the overall meta-analyses suggest that PSMs do affect diet selection in wild primates, this conclusion is driven mostly by a significant effect of condensed tannins on the diet choice of colobine primates. No other PSMs were found to influence diet selection in colobine primates. Furthermore, there was no significant effect of any PSM on non-colobine primates when considered as a single group. Therefore, we suggest that there is evidence that colobine

primates exhibit aversion to condensed tannins, but insufficient evidence to date to conclude that primates in general exhibit diet selection choices in response to PSMs. Although we found this overall trend, there is certainly diversity among different colobine species with respect to the role of tannins on food selection (Rothman et al., 2022). Early studies noted that some forests had more PSMs in their leaves than others; west African and Asian forests contained higher concentrations of condensed tannins in their leaves compared to east African forests (Oates et al., 1990). This difference likely affects food choices; in Kibale National Park, Uganda, mature leaves did not differ in tannin concentration from young leaves (Chapman & Chapman, 2002), and red colobus monkeys (*Procolobus rufomitratus*) selected young leaves, suggesting a different selection criterion. However, the sympatric guereza (*Colobus guereza*) in the same forest did appear to select leaves based on condensed tannins (Oates et al., 1977). A later study of tree use by *Colobus guereza* in the same area did not find that condensed tannins limited the time spent in trees, but instead that macronutrient balance was important in this respect (Johnson et al., 2017). Condensed tannin concentrations in this forest are lower than in other areas (Oates et al., 1990), indicating it is important to consider the nature of the PSM, the microhabitats of the primates under study, and the nutritional environment in these considerations.

We found no evidence of a consistent effect of PSMs in influencing the feeding choices of non-colobine primates in general. However, it is important to note that the "non-colobine" group is an extremely diverse group of 10 subfamilies/families from lemurs to apes, and this diversity of behavioral responses and adaptations to PSMs are not accounted for in our analysis. There are many reasons why we would not expect to find a consistent effect of PSMs on this diverse group. For example, there are many examples where non-colobine primates select for higher concentrations of PSMs, presumably for self-medication (Huffman, 1997, 2003; Morrogh-Bernard et al., 2017), phytoestrogens (Wasserman et al., 2012) to control mineral absorption (Simmen et al., 2006; Spelman et al., 1989), or for their antiparasitic properties (Carrai et al., 2003; Rothman, Pell, et al., 2009). Further, there may be an advantage to different tolerances and preferences for PSMs among primate communities. For example, Ganzhorn (1988, 1989; Ganzhorn et al., 1985) found that individual species of a lemur community showed distinct tolerances to PSMs and proposed that this contributes to niche separation in sympatric primate communities.

Despite the diversity of the group, the responses (effect size) within each subfamily were highly variable. Importantly, studies with reasonably high precision tended toward smaller effect sizes and conversely, studies with low precision tended to have large effects (Figure 2). The heterogeneity among effects (I^2) for species broadly represents the "ecological/environmental" aspects of taxonomy, that is, the habitat the species lives in and its behaviors. I^2 for species was relatively small (26.9%, Table 3), suggesting that there is not likely to be any single trait to explain the variation in effects. Therefore, including families or subfamilies of non-colobine species as covariates (moderators) can at very best explain only ~30% of the variation and

will most likely dilute the effect of individual species, the true source of variation. We explicitly included species and phylogeny in the multilevel analysis as random factors, rather than fixed factors, for two reasons. First, without a strong a priori hypothesis as to why specific clades should be different (e.g., different digestive anatomies or strategies), then it is hard to justify including them as fixed factors. Second, the number of effects per species was insufficient to include phylogeny or species as fixed factors. Therefore, explaining variation in responses to PSMs within the non-colobine primates will require more studies, and for those studies to be undertaken with far more precision than has been done previously.

4.2 | Meta-analyses: Methodological problems

There are inherent problems in meta-analyses. We caution that the data used in these analyses were very limited, uneven, and in many ways flawed because of the methods used for the analysis of PSMs. In some cases of potential interest, there were so few data points (e.g., effect of alkaloids) that no reliable conclusions could be drawn. In other cases where the analysis methods for PSMs is more reliable (e.g., cyanogenic glycosides), the data provided in the published studies were insufficient to calculate effect sizes. Thus, major groups of PSM likely to be important to primates were not included in the analysis. Furthermore, the imbalanced coverage of species and primate groups means that results from these analyses must be used cautiously and the limitations of the data recognized. For example, the analyses should not be cited as proof that colobines are more susceptible to PSMs than other primates without additional well-designed and statistically robust studies that include the diversity of other primate groups. Rather, we hope the analyses presented will be informative in driving improvements in future studies.

4.3 | Statistical issues

The assumption of independence among data points holds for meta-analytical methods, the same as for conventional statistical analyses. Nonindependence can arise from many sources, and these need to be addressed to generate a meaningful estimate of the true variation in effect sizes. While issues related to nonindependence due to repeated measurements within a study, and between-study effects are well known, methods for dealing with the relatedness among species are relatively recent. Multiple measurements of the same species as well as the phylogenetic relatedness among species can be, at times, a significant source of error in meta-analyses involving a wide range of taxa (Chamberlain et al., 2012). Indeed, we observed that multiple measurements on the same species had a significant effect on the proportion of heterogeneity among effect sizes, as evidenced by the relative magnitude of I^2 coefficient indicating the proportion of heterogeneity attributable to repeated measurements on the same species. Conversely, the phylogenetic signal was generally small or nonexistent. This is not surprising, given the relatively short evolutionary history of the primate

clade. There were 43 primate species in our data set with multiple studies of some species (e.g., 15 effect sizes for *Alouatta palliata*; 10 effect sizes for *Macaca mulatta*, and eight studies of *Presbytis melalophos*), but an uneven coverage across taxa. Three species were excluded from the multilevel meta-analysis models, due to not being readily resolvable within the phylogeny (*Calicebus melanochir*), or extremely large values (*Chlorocebus pygerythrus* and *Lophocebus albigena*). The inclusion of these species, however, would not be expected to materially influence the broader interpretations from this analysis. Furthermore, the limited number of effect sizes for multiple species (e.g., single effect size for multiple species, Supporting Information: Table S1) makes interpreting species-level responses challenging. Rather, we suggest that variation among species is more readily interpretable, rather than individual-level responses (Figure 3).

Many studies that were included in this analysis had small sample sizes, which in turn leads to low precision in estimates of effect size, that is, large effect sizes and relatively large variances. Conversely, those studies with larger sample sizes and greater precision tended toward relatively small effect sizes, suggesting that the true effect of PSMs on primate feeding behavior could be small, though there are additional caveats in the analytical methods used. Many studies have previously relied on nonparametric tests, seemingly to overcome issues of unbalanced designs and heterogeneity in variances between comparison groups within studies. Obtaining sufficient sample sizes in behavioral studies is constrained by a variety of practical and ethical factors, particularly when working with threatened and rare species (Garamzegi, 2016). However, our results highlight that to achieve better scientific precision and certainty in understanding the influence of PSMs on primate feeding, future efforts should be invested in more robust study designs with greater consideration of appropriate experimental designs and statistical power.

Other methodological issues were highlighted in this analysis that require consideration in future studies. We found a significant effect of PSMs in studies that examined food items that were eaten compared to plant parts that were not consumed, but no significant effect of PSMs in studies that examined PSMs along a preference gradient of foods consumed by focal primates. There are a multitude of food components in addition to PSM concentration that are likely to influence primate food choice, including energy, protein, and fiber (Eppley et al., 2017). Further, obtaining a sufficient sample size is expected to be particularly problematic for studies examining a preference gradient, compared to a binary comparison of eaten foods versus not eaten foods. As all foods were consumed within the variable preference studies, it is plausible that foods with higher PSM concentrations were not consumed at all; hence, did not factor into these studies, greatly limiting the ability to detect an effect of PSM concentration in relation to food choice.

4.4 | Nutritional ecology of wild primates

A recent meta-analysis of studies of wild primate feeding choices in relation to protein showed that soluble protein and acid detergent fiber were limiting factors in primate feeding choice, but not total protein (Ganzhorn et al., 2017). The authors found that the selection for soluble protein was stronger when the average concentration of protein in leaves from a representative sample of the habitat was low, but that preference for fiber was not related to fiber in the representative sample (Ganzhorn et al., 2017). The interaction between primate feeding behavior and habitat quality may explain why we did not find a stronger effect of PSMs on primate feeding, particularly among non-colobine species. It is possible that primates only act to regulate PSM intake when they regularly encounter high concentrations of PSMs or when protein is limited, as they may be closer to their limit for PSM detoxification compared with others in higher quality habitats. Future studies into primate feeding ecology, particularly studies that compare results between sites and in different seasons, should consider the nutritional properties of the habitats using measurements of representative samples (Ganzhorn et al., 2017; Rothman et al., 2012).

One promising recent approach is to use *in vitro* assays to estimate the effects of PSMs on the availability of protein. DeGabriel et al. (2009) drew on agricultural approaches to develop a simple assay to measure the effects of tannins and fiber on the availability of protein and showed that common-brushtail possums (*Trichosurus vulpecula*) in home ranges with higher available protein had greater reproductive success and faster growing offspring than those in poorer quality areas (DeGabriel et al., 2009). This method has also been used to yield new insights into primate feeding ecology (Droescher et al., 2016; Evans et al., 2021; Felton et al., 2009) and could be easily incorporated into nutritional ecology studies.

4.5 | Digestive physiology of colobines

Although the digestive physiology of primates varies widely (Chivers, 1994; Chivers & Hladik, 1980; Cork & Foley, 1991; Lambert, 1998), a major contrast for the tolerance of PSMs is the difference between the foregut-fermenting colobines and other species which ferment refractory materials in the hindgut. Microbial metabolism of ingested PSMs can facilitate structural changes that can lead to either less toxic products or in some cases, more toxic products (Carlson & Breeze, 1984; Duncan & Poppi, 2008; Foley et al., 1999; Freeland & Janzen, 1974; Kohl et al., 2014). In addition, ingested compounds with known antimicrobial effects have been predicted to be deleterious to foregut-fermenting species (Duncan & Poppi, 2008; Wallace, 2004). The outcomes of the interactions between the microbiome and PSMs are compound-dependent and it is not possible to make reliable predictions of their effect without detailed chemical and metabolic data (see Blyton et al., 2019; Kohl et al., 2014). Nonetheless, we found no evidence from the existing data that colobine primates were at a significant advantage over non-colobines in consuming diets rich in secondary metabolites as has been proposed in the past (Freeland & Janzen, 1974; Kay & Davies, 1994; Mowry et al., 1996).

In contrast, our finding that colobines were deterred by condensed tannins opens the door for a range of new studies on the role of tannins in primate nutrition and health. Studies in other

foregut-fermenting herbivores (mainly ruminant livestock) have shown that although tannins can depress protein digestibility (e.g., Robbins et al., 1987), low concentrations may be beneficial since they can protect high-quality protein from degradation in the foregut. Whether these effects could occur in colobines depends on unresolved questions about digesta flow in the foregut of colobines and specifically, the extent to which ingesta could escape extensive fermentation (Cork, 1996; Lambert, 1998; Schwarm et al., 2009). Similarly, tannins in the diets of domestic ruminants have also been shown to provide beneficial effects against parasitic helminths (Mueller-Harvey et al., 2019) and we have already highlighted (above) the possibility that the same occurs in some wild primates.

The foregut anatomy of colobines has been described by Langer (1988) as being either tripartite or quadripartite wherein some species have an additional chamber called the praesaccus. Matsuda et al. (2019) have suggested that the praesaccus is associated with species more reliant on foliage (in which tannins are likely more common), whereas the tripartite condition is more likely in species consuming seeds and digestible fruits. Nonetheless, we found that both groups of colobines were deterred by condensed tannins in the diet. With a greater diversity of species and detailed chemical data on the diet, this finding could be explored in more detail in the future.

4.6 | Structure and diversity of PSMs

The huge diversity of chemical structures of PSMs eaten by mammalian herbivores has led to many hypotheses about their modes of action in deterring feeding. Although none of these hypotheses have been addressed directly in studies of primates, the chemical structure clearly has the potential to explain variation in the effect of PSMs on feeding in folivores (Lawler et al., 1999; Marsh et al., 2015).

Understanding the consequences of differential activity of PSMs requires detailed chemical data. Similarly, the structure of tannins predictably determines their activity in standard assays (Engström et al., 2019; Salminen & Karonen, 2011). Tannins are likely to be encountered more frequently than alkaloids by herbivores in leaves and fruits and so specific countermeasures may have evolved to minimize deleterious effects, such as salivary tannin-binding proteins (Shimada et al., 2011). Nonetheless, understanding and building on the finding that condensed tannins deter feeding in colobines requires better knowledge of the structure, and concentration of tannins in the diet together with a better knowledge of the diet composition. Continuing to rely on a single assay and single standard within that assay to quantify condensed tannins in tree leaves denies the extensive work in the past 20 years that shows that the chemical structure of tannins matters as well as concentration.

4.7 | Where to from here?: Metabolomics

Primatology is at a crossroads in terms of understanding the role of PSMs in primate diets. The analysis we have presented indicates the

potential of significant ecological effects including critical interactions between the digestive physiology of animals and the effectiveness of different classes of PSMs in food choice. However, in the future, we believe that it is time for primatologists to abandon the broad nonspecific assays such as "total phenolics" and reliance on colorimetric analyses of "alkaloids" and re-engage with phytochemists to better characterize the chemical profile of food plants. Over the past generation, however, natural product chemistry has declined as a discipline in most western universities and collaborations between primatologists and phytochemists have diminished except in studies of self-medication (e.g., Ohigashi et al., 1991) analysis.

There has also been an enormous improvement in methods for the high throughput separation and (at least partial) characterization of plant metabolites. The discipline of metabolomics has grown to complement other "-omics" technologies and it is now realistic to attempt to understand the diversity of chemical composition in hitherto uncharacterized tropical plants (Coley et al., 2018; Salazar et al., 2016; Sedio et al., 2018; Sedio, 2017). Although structural characterization of ecologically significant secondary metabolites should be the ultimate goal, matrices of similarity are also useful (Sedio et al., 2017), particularly when combined with the detailed feeding data that primatologists have excelled in collecting. Metabolomic approaches have recently started being applied to primate feeding (Amato et al., 2017; Garber et al., 2019) but, to date, there has been no specific focus on PSMs. Given that primates often consume very diverse diets, using an approach such as examining matrices of chemical similarity (Sedio et al., 2021) could prove useful in identifying the diversity of compounds these generalist primates encounter.

Although chemical data are essential in understanding the types of compounds that animals consume, linking chemistry with functional effects in animals is essential if we are to predict ecological impacts now and in the future. For example, the functional measure of "available protein" described above has now been demonstrated to be related to structural features of the tannins in *Eucalyptus* foliage (Marsh et al., 2020) (i.e., the proportion of pro-delphinidin subunits in condensed tannins as well as their mean degree of polymerization). Similarly, although flavanones are common in *Eucalyptus* foliage, only those with an unsubstituted B-ring are a deterrent to a marsupial, the common brushtail possum (Marsh et al., 2015).

Much of the progress in other taxa in understanding the roles of secondary metabolites in feeding ecology has been based on captive studies where diets can be manipulated, microbial populations modified, and interactions between PSMs and nutrients quantified (DeGabriel et al., 2014). Because diets ex situ can be easily manipulated and controlled (Marsh et al., 2020), it is easier to recognize the effects of specific PSMs. Conversely, these types of experiments are usually not possible with most wild primates (Espinosa-Gómez et al., 2018). Additionally, captive diets typically cannot represent the diverse array of secondary compounds that primates may encounter in their habitats, and their foraging choices may depend on an array of factors (competition with sympatric animals and fear of predation), such that an acceptable food in one scenario would not be acceptable in a captive environment. As such

there are also limitations in the interpretations of diet experiments in captivity.

However, habituated groups of primates can be a reliable source of excreta (both feces and urine) (Gillespie, 2006; Rangel-Negrín et al., 2009; Rothman et al., 2008) which may allow the functional significance of ingested PSMs to be evaluated. For example, the role of tannin-binding salivary proteins has been studied in a few primates (Espinosa-Gómez et al., 2018; Mau et al., 2009, 2011) but the functional significance of these countermeasures against dietary tannins remains unknown. Shimada et al. (2011) has shown that fecal concentrations of proline (derived from proline-rich salivary proteins) correlated strongly with dietary tannin intake in wild rodents. Approaches such as these could be applied to collections of excreta from wild primates and matched with longitudinal data on tannin intake in different species. This could allow testing of questions around whether tannins contribute to dietary differences between sympatric species.

Metabolomic approaches can be applied to urine to identify markers of metabolism of ingested secondary metabolites. For example, a well-characterized drug metabolism pathway in humans is that mediated by cytochrome P450 (CYP2D6). This enzyme has a very strong affinity for alkaloids (Fonne-Pfister & Meyer, 1988) and metabolizes a large proportion of human drugs. Tay-Sontheimer et al. (2014) used untargeted metabolomics to identify a metabolite in the urine of human infants that was correlated with the activity of CYP2D6. An approach like this might allow testing of questions as to why some species of lemurs are apparently indifferent to alkaloids (Ganzhorn, 1988). There is a significant effort toward finding markers for the activity of other important human CYP enzymes (Magliocco et al., 2019) that might provide a way of studying the metabolism of PSMs in wild primates. Combining primate feeding data with metabolomics of excreta collected in the wild could be a powerful way to study PSM metabolism in primates.

4.8 | Why are PSMs important and why do we need better designed studies?

We need to explore the potential role that plant defensive chemistry plays in structuring primate communities, driving distributions, and survival. DeGabriel et al. (2014) outlined four steps for linking the feeding behaviors of individuals with population- and species-wide outcomes, which also summarizes the lessons we can draw from this meta-analysis for future studies of primate feeding behavior: (1) knowing the quality and amount of foods eaten and avoided, (2) choosing appropriate measures of plant chemistry and effective ways to measure them in a particular system; (3) understanding the variation in nutritional quality and potential nutritional limitations of habitats, and (4) using and reporting appropriate statistics.

Linking feeding behaviors of individuals with population-wide metrics is particularly pressing because recent work in other groups of mammals has demonstrated major interactions between ambient temperature and the ingestion of foods rich in secondary metabolites

(Beale et al., 2018; Dearing, 2013). Diets eaten by folivores have a particularly high cost of diet-induced thermogenesis, or the heat increment of feeding (HIF) arising from the thermogenic cost of detoxification (Beale et al., 2018). Warmer temperatures, even temperatures within the herbivore's prescriptive zone (Youngentob et al., 2021), increase toxicity and reduce an animal's dietary options, and can even lead animals to reduce or avoid feeding altogether to ensure that the energy increment of digestion does not contribute additional heat (Beale et al., 2018; Dearing, 2013; Kurnath et al., 2016). There is evidence that PSM detoxification consumes considerable protein (Au et al., 2013), raises metabolic rate, and may directly interfere with the ability of mammals to maintain a constant body temperature due to a decrease in liver and mitochondrial function leading to greater heat production (Beale et al., 2018; Dearing, 2013). It is clear that even small changes in ambient temperature can lead to major changes in the feeding patterns of browsing herbivores (Beale et al., 2018; Youngentob et al., 2021). Furthermore, increased atmospheric CO_2 concentrations can reduce the concentration of protein, increase fiber and increase the concentrations of toxins in leaves (Robinson et al., 2012; Rothman et al., 2015).

A more rigorous approach to incorporating PSMs into studies of primate feeding ecology is critical in predicting how animals will respond to global warming and other environmental change (Bernard & Marshall, 2020; Stalenberg, 2019). Over the past decade, researchers have developed species-specific mechanistic models to predict the response of species to climate change (M. Kearney & Porter, 2009; M. R. Kearney et al., 2010). These models simulate the physiological and behavioral response of individual animals to environmental change to predict survival, movement, distribution, and risks of extinction under a great variety of future climate and habitat scenarios (Briscoe et al., 2016; Mathewson et al., 2017). These modeling approaches will not be complete without a better understanding and integration of the factors that influence the feeding ecology and nutritional physiology of primates, and especially the impacts of PSMs under changing conditions.

5 | CONCLUSION

We urge primatologists to connect with phytochemists to develop targeted techniques, investigate established and emerging metabolomic techniques to understand chemical diversity in the diet of primates and use more robust statistical designs and analyses. PSMs are likely to be critical mediators of feeding in many primate species since other mammal−plant systems, where more detailed chemical work has occurred, have all shown major effects at the individual and population levels. More rigorous approaches to incorporating PSMs into studies of primate feeding ecology are critical if the field is to adapt to meet the challenges of the future.

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DATA AVAILABILITY STATEMENT

The paper is a review of previously published data. The R code used for the meta-analysis of these published data is available from the corresponding author on reasonable request.

ETHICS STATEMENT

This paper reports on the statistical analysis of existing published literature on feeding in primates. No new fieldwork was conducted and no unpublished data were included in the analyses.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.