Gametocyte production and transmission fitness of African and Asian *Plasmodium falciparum* isolates with differential susceptibility to artemisinins

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Abstract

The emergence of *Plasmodium falciparum* parasites partially resistant to artemisinins (ART-R) poses a significant threat to recent gains in malaria control. ART-R has been associated with PfKelch13 (K13) mutations, which differ in fitness costs. This study investigates the gametocyte production and transmission fitness of African and Asian *P. falciparum* isolates with different K13 genotypes across multiple mosquito species. We tested three ART-sensitive (ART-S) isolates (NF54, NF135, NF180) and three ART-R isolates (ARN1G, 3815, PAT-023) for sexual conversion and transmission to *Anopheles stephensi, An. gambiae* and *An. coluzzii*. ART-R levels were quantified *in vitro* using the Ring-stage Survival Assay (RSA), and the transmission-reducing effects of dihydroartemisinin (DHA) on mature gametocytes were assessed. Results showed that ART-S parasite lines consistently produced gametocytes and transmitted effectively in all three mosquito species. ART-R isolates showed variability: ARN1G maintained high transmission levels, whereas 3815 showed limited transmission potential despite higher sporozoite loads in *An. coluzzii*. The African ART-R isolate PAT-023 demonstrated low gametocyte commitment but was transmitted efficiently in both *An. gambiae* and *An. coluzzii*. DHA exposure reduced mosquito infectivity for all isolates, regardless of K13 genotype. These findings, based on a limited number of field isolates, suggest that ART-R parasites remain transmissible across different *Anopheles* species. However, K13 mutations do not appear to confer a direct transmission advantage. This study highlights the complexity of ART-R dynamics and underscores the need for further research to inform malaria control strategies in regions where ART-R parasites are circulating.

Key words

Malaria, *P. falciparum*, Mosquito Transmission, Artemisinin Resistance, K13 genotype

48 ## Introduction

49 Despite global efforts to reduce malaria burden, progress has plateaued in the last decade and in
50 some areas malaria is again increasing [1]. The recent emergence in Sub-Saharan Africa of *P.*
51 *falciparum* parasites with partial resistance to artemisinins (ART-R) adds to the concerns about how
52 sustainable malaria control may be. ART-R is characterised by prolonged parasite clearance times
53 (half-life >5 hours) or persistence of parasitemia on day 3 following artemisinin monotherapy or
54 artemisinin-based combination therapy (ACT) [2-6]. ART-R is predominantly associated with specific
55 mutations in the essential PfKelch13 (K13) protein's β-propeller domain [7-10]. These mutations
56 reduce K13 protein levels, disrupting haemoglobin import into the parasite's digestive vacuole, a
57 critical step for ART activation [8, 11-13]. The impacts of K13 mutations on both resistance and
58 parasite fitness are variable, depending on the mutation and parasite background [13-16], which
59 makes it difficult to predict which mutations are of particular concern.

60 Clinically relevant mutations in K13 first were observed in isolates collected in 2002 in western
61 Cambodia [17]. While partial resistance subsequently spread across the Greater Mekong Subregion
62 [7, 18], the anticipated expansion to the African continent has not occurred. It was hypothesised that
63 lower drug pressures combined with high parasite diversity in many settings in Sub-Saharan Africa
64 may have allowed wild-type parasites to outcompete less-fit K13 mutant parasites, making *de novo*
65 emergence of ART-R less likely and limiting the spread of the K13 mutants upon introduction. The
66 recent independent emergence of K13 mutations associated with ART-R including in East Africa
67 (Uganda, Rwanda, and Tanzania) and the Horn of Africa (Eritrea, Ethiopia and Sudan) [19-23] raises
68 questions about their fitness, resistance, and transmission potential. Transmission to mosquitoes
69 depends on the formation of viable male and female gametocytes, with considerable variation in
70 gametocyte production between parasite isolates [24, 25]. The transmission potential of parasites
71 with K13 mutations is particularly relevant with changes in vector populations and variation in *P.*
72 *falciparum* vector competence [26]. The recent invasion of the competent Asian vector *Anopheles*
73 *stephensi* in urban African settings [27, 28] raises concerns about the spread of ART-R parasites of
74 African and Asian origin in settings previously not endemic for malaria.

75 Understanding variations in vector competence and determining whether *K13* mutant parasites
76 possess a transmission advantage, both in the presence and absence of artemisinin derivatives, are
77 crucial for predicting the potential spread of ART-R on the African continent. In this study, we
78 investigate the gametocyte production and transmission fitness of ART-S and ART-R field isolates. We
79 compared ART-S isolates [29, 30] with ART-R isolates carrying two common Southeast Asian K13

80 mutations G449A and C580Y [18, 31, 32], as well as a recently isolated ART-R parasite line from
81 Uganda, where the local emergence of ART-R has been documented.
82

83 Material and methods

84 **Parasite cultures**

85 All parasite lines – including the ART-S NF54 (Africa), NF135 (Cambodia) and NF180 (Uganda), as well
86 as the ART-R *P. falciparum* field isolates ARN1G (Thailand), 3815 (Cambodia), and PAT-023 (Uganda)
87 – were cultured in an automated culture system using RPMI media supplemented with 10% human
88 serum [33]. Asexual parasite cultures were maintained in a synchronised state through magnet
89 separation or sorbitol lysis. Gametocyte induction was performed using either minimal fatty acid
90 media or 0.5% Albumax; gametocytes were allowed to mature in RPMI media supplemented with
91 10% human serum [25]. Parasite DNA was extracted with QIAGEN Blood DNA kit, sequenced on the
92 Illumina Novaseq 6000 platform and analysed using the malaria profiler tool [34].

93 **Ring-stage Survival Assay (RSA)**

94 The Ring-stage Survival Assay (RSA) was performed as previously published [16, 35] with minor
95 modifications. To avoid the negative impact of sorbitol synchronisation on conversion rates within
96 the same cycle [36, 37], we opted for a gentler approach using double magnet purification. Highly
97 synchronised parasites were first passed through a magnetic column (MACS) to collect segmented
98 schizonts. These schizonts were put back into culture for 3-4 hours to allow bursting and reinvasion
99 of new red blood cells (RBCs). The culture was subsequently passed through the same magnetic
100 column, isolating the flow-through, which contained only newly invaded ring-stage parasites.
101 Parasites were split into 2 plates: one for the sexual conversion assay and one for the RSA. For the
102 RSA, parasites were diluted to 1-2% parasitemia in 2% hematocrit. Each line was tested in 4 wells:
103 two for DMSO controls and two for DHA. Washes were performed in separate tubes following DHA
104 treatment before being added to a fresh plate. Survival rates were determined using both Giemsa
105 smears and flow cytometry using MitoTracker Red as a live cell stain.

106 **Sexual conversion assay**

107 Sexual conversion rates were performed as previously published [25], with minor modifications.
108 After double synchronisation, parasites were returned to culture for 24 hours prior to initiating the
109 conversion assay. The assay was performed in a plate-based format with each parasite isolate split
110 over 3 wells (one for each media type) at 1% parasitemia in 5% hematocrit. Conversion rates were
111 calculated by Giemsa-stained smears [25].

112 **Mosquito infections**

113 Laboratory colonies of *An. stephensi* (Nijmegen Sind-Kasur strain)[38], *An. coluzzii* (N'gousso strain)
114 [39] and *An. gambiae* s.s (Kisumu strain) [40], were maintained under controlled conditions: 26°C,
115 70-80% humidity and a 12-hour reverse day/night cycle. Groups of 100 female *Anopheles*
116 *stephensi*, *An. gambiae*, and *An. coluzzii* mosquitoes, aged 1–5 days, were blood-fed for 15 minutes
117 using glass membrane mini-feeders (15 mm diameter, convex bottom) connected to a heated
118 circulating water bath. Fully fed mosquitoes were selected, and maintained at 30°C with access to 5-
119 10% glucose.

120 **Ookinetes count**

121 Mosquito midguts were examined 18-24 hours post-infection to identify the presence of round
122 forms, retort forms, and mature ookinetes. Five midguts from each infection group were dissected
123 and incubated with a 1:50 diluted Anti-25KD-FITC conjugate in Evans blue solution. Midguts were
124 gently disrupted using a pipette tip to release the blood meal. Following incubation in the dark for 30
125 minutes at room temperature, the solution was washed with 1.4 mL of phosphate-buffered saline
126 (PBS), vortexed to dissolve the pellet, and centrifuged for 2 minutes at 10,000 rpm. After removal of
127 the supernatant, the pellet was resuspended in 25 µL of PBS and 5 µL of the suspension was loaded
128 into a Bürker-Turk counting chamber. Round forms, retort forms and mature ookinetes were counted
129 using an incident light fluorescence microscope with a GFP filter at 400× magnification.

130 **Oocysts count**

131 Seven days post-infection, 20 mosquito midguts per group were dissected, stained with 1%
132 mercurochrome, and examined under an optical microscope at 100× magnification to detect and
133 quantify oocysts.

134 **Sporozoites count**

135 Mosquitoes used to examine sporozoite development received a second (uninfected) bloodmeal on
136 day 4-6 post infection to synchronise oocyst development Fifteen days post-infection, mosquito
137 salivary glands were dissected in PBS and transferred to oocyst lysis buffer (NaCl 0.1M: EDTA 25mM:
138 TRIS-HCl 10mM). Following overnight incubation at 56°C with Proteinase K, DNA was extracted with
139 the automated MagNA Pure LC instrument using the MagNA Pure LC DNA Isolation Kit – High
140 performance. Sporozoite density was analysed using COX-I qPCR [41].

141 **Statistical analysis**

142 Statistical analyses were conducted using R software (v 3.1.12) [42]. Mean counts of oocysts,
143 sporozoites, and ookinetes (with 95% confidence intervals) were estimated using a mixed-effects
144 negative binomial regression model, incorporating random intercepts for biological replicates and

145 fixed effects for parasite line, mosquito species, and their interactions. Mean survival and
146 commitment rates (with 95% confidence intervals) were calculated using a mixed-effects beta
147 regression model. Transmission-reducing activity (TRA) and transmission-blocking activity (TBA)—
148 reflecting reductions in oocyst density and the proportion of infected mosquitoes, respectively—
149 were assessed for 700 nM and 7000 nM DHA using a Bayesian Poisson regression model.

150

151 ## Results

152 **Parasite Backgrounds**

153 We used well-characterised *P. falciparum* reference lines from Africa (NF54), Southeast Asia (NF135,
154 Cambodia) [29], and a new isolate from East Africa (NF180, Uganda). These lines were
155 complemented with previously published ART-R K13 mutant parasite lines from Thailand (ARN1G,
156 K13-449A) [32], from Cambodia (3815, K13-580Y) [31, 35] and a recently collected isolate from
157 Uganda with *in vitro* ART-R that is independent of K13 mutations (PAT-023). Genotyping revealed
158 that some isolates harboured mutations associated with resistance to chloroquine and sulfadoxine-
159 pyrimethamine (Table 1).

160 **Artemisinin resistance**

161 ART-R lines ARN1G and 3815 lines showed survival rates in the RSA that were higher than ART-S
162 NF54, as previously published ([16, 32, 35];Figure 1a, Sup Table 1). The ART-S NF135 line showed
163 variable survival rates, which were lower than those observed for known ART-R lines but higher than
164 those observed for ART-S NF54 and NF180 lines. The PAT-023 parasite line exhibited a survival rate of
165 9.1% (95% CI: 6.6% - 12.5%), higher than the ART-S lines.

166 **Sexual conversion**

167 We assessed the ability of ART-R and ART-S lines to convert under two different conditions that
168 mimic the natural sensing system [25, 43, 44]. All conversion assays were performed on a single
169 asexual cycle. The highest rates of gametocyte conversion were typically observed using minimal
170 fatty acid media with intermediate conversion rates for media containing Albumax only, and lowest
171 conversion in non-inducing serum media (Figure 1b, Sup Table 2) [25]. The conversion rates observed
172 for K13 mutants were comparable to those recorded for ART-S lines, except for the Cambodian line
173 3815, which exhibited highly variable conversion rates (Figure 1b). The Ugandan ART-R line PAT-023
174 exhibited minimal sexual conversion with little difference between media types (Figure 1b, Sup Table
175 2).

176 **Transmission to mosquitoes**

177 Transmission was examined using three mosquito vector species. We first assessed whether
178 fertilisation occurred in mosquitoes for each parasite isolate by quantifying parasite developmental
179 stages in the mosquito midgut 20-24 hours post-infectious blood meal. We distinguished between
180 mature and immature stages following Pfs25 antibody labelling. Immature stages included
181 underdeveloped ookinetes and rounded forms classified as either zygotes or unfertilised females.
182 The ART-S parasite isolates NF180 and NF135 showed no clear difference in the numbers of
183 immature or mature ookinetes across the mosquito species tested (Figure 2a). For NF54, there was a
184 tendency for fewer mature ookinetes in *An. gambiae* compared to *An. stephensi* and *An. coluzzii*,
185 though this was not statistically significant (Figure 2a). A similar trend was observed with the ART-R
186 ARN1G parasite line. For the Ugandan ART-R isolate PAT-023, significantly fewer mature ookinetes
187 were observed in *An. stephensi* (Figure 2a) compared to *An. coluzzii* ($p = 0.019$). However, this
188 significance level should be interpreted cautiously due to the number of comparisons made. The
189 ART-R isolate 3815 consistently produced low numbers of mature ookinetes in all *Anopheles* species
190 tested (Fig 2a).

191 To confirm that mature ookinetes can establish mosquito infections, we quantified oocyst prevalence
192 (Sup Figure 1) and density (Figure 2b) on day 7 post-bloodmeal. African parasite lines (NF54, NF180,
193 and PAT-023) exhibited higher oocyst densities in traditional African mosquitoes (*An. gambiae s.s.,*
194 *An. coluzzii*) compared to *An. stephensi,* particularly for PAT-023 ($p < 0.0001$; Sup Table 3). In contrast,
195 NF135 showed consistent infection intensities across all tested mosquito species. For the Asian K13
196 mutant lines, oocysts densities in *An. stephensi* were slightly higher compared to *An. coluzzii* and *An.*
197 *gambiae*. Specifically, K13 mutant parasite lines ARN1G had higher densities in *An. stephensi* relative
198 to *An. coluzzii* ($p = 0.009$) and *An. gambiae* ($p < 0.0001$), while 3815 showed similar trends ($p = 0.007$
199 compared to *An. coluzzii* and $p<0.0001$ compared to *An. gambiae*; Sup Table 3). The 3815 isolate
200 exhibited low and highly variable infection levels, consistent with its reduced mature ookinete counts
201 (Figures 2a and 2b). Interestingly, a trend towards higher sporozoite production was observed in the
202 two K13 mutant lines (ARN1G and 3815), which persisted even after normalising for oocyst density
203 within the same batch of mosquitoes. However, transmission results for the 3815 isolate remained
204 highly variable (Sup Figure 2).

205 **Transmission-blocking effect of DHA**

206 With all parasite lines being able to infect mosquitoes in the absence of drug pressure, we
207 determined the transmission-reducing effect of DHA on mosquito infection intensity and prevalence.
208 Mature gametocytes were exposed to both the physiologically relevant concentration of 700nM and
209 a tenfold higher concentration of 7000nM for 48 hours before mosquito feeding with the drug not
210 removed before mosquito feeding [32, 45]. Given the labour-intensive nature of these assays and the

211 known permissiveness of *An. coluzzii* for the parasites tested herein, experiments were performed
212 exclusively with this mosquito species. All isolates showed a consistent decrease in mosquito
213 infection intensity and prevalence as DHA concentrations increased, independent of the K13
214 genotype and the parasite background (Figure 3, Sup Figure 3). At 7000nM DHA, sporadic infections
215 were observed for the African isolates NF54, NF180, and PAT-023, but none of the Asian isolates
216 infected mosquitoes at this concentration. When relative reductions in oocyst intensity were
217 calculated, DHA exposure at 7000nM reduced oocyst intensities by >93% for all parasite isolates.
218 There were no indications of reduced transmission-blocking efficacy of DHA for ART-R isolates.

219 **Correlating resistance and transmission stages**

220 Although the number of parasite isolates examined was modest, our data on asexual parasite
221 survival under DHA pressure, combined with findings on sexual conversion and mosquito
222 transmission, provide an opportunity to investigate whether ART-R confers a transmission
223 advantage. We initially hypothesised that parasites with high levels of *in vitro* resistance to ART at
224 the ring stage would show enhanced transmission to mosquitoes under drug pressure. However, no
225 correlation was observed between parasite survival in the RSA and the reduction in oocyst density
226 under DHA pressure (Figure 4a). In contrast with our initial hypothesis, we observed a weak negative
227 correlation between parasite survival rates in the RSA and gametocyte conversion rates upon
228 induction (Figure 4b).

229

230 Discussion

231 Understanding the transmission fitness of parasites that survive ART-based treatment is important
232 for developing strategies that aim to prevent or slow down the spread of resistance. Some reports
233 have suggested that K13 mutant parasites may have higher intrinsic gametocyte production [4, 46],
234 increased survival of specifically male gametocytes under DHA exposure, and larger oocysts in the
235 mosquito midgut [32].

236 We selected three parasite isolates from Southeast Asia and three from Sub-Saharan Africa, each
237 with distinct K13 genotypes and genetic backgrounds. The Asian ART-R lines ARN1G and 3815
238 exhibited higher parasite survival in the RSA, consistent with previous studies [16, 32, 35]. PAT-023, a
239 newly characterised Ugandan ART-R line that is wild-type for K13, demonstrated a high level of
240 survival in the *in vitro* RSA. Interestingly, we also observed that NF135, a line previously associated
241 with treatment failure following artemether-lumefantrine therapy *in vivo* [47], showed erratic and
242 occasionally increased DHA survival rates.

243 This study focuses on the transmission potential of *P. falciparum* isolates, beginning with their
244 commitment to sexual stages. Fatty acids play a key role in signalling the parasite to commit to sexual
245 conversion [36, 37]; we used two methods targeting this signalling pathway to robustly compare
246 sexual conversion rates. From all media conditions tested, minimal fatty acid media consistently
247 produced the highest sexual conversion rates across all lines. Among the two ART-S parasite isolates,
248 NF54 and NF135 consistently exhibited high conversion levels. The newly introduced NF180 line,
249 which has not been characterised in detail previously, demonstrated high conversion rates,
250 comparable to NF54 and higher than NF135. Among the ART-R parasite lines, PAT-023 displayed the
251 lowest commitment to gametocyte production, while ARN1G showed commitment levels similar to
252 NF135. The Cambodian 3815 line exhibited an inconsistent commitment to gametocyte production,
253 with occasional high levels that decreased with prolonged culture, potentially reflecting epigenetic
254 silencing during *in vitro* cultivation.

255 We observed no evidence of increased of sexual conversion in the ART-R isolates. While sexual
256 conversion is a critical step for transmission, high commitment rates do not always correlate with
257 high mosquito infectivity [25, 29]. The presence of mature ookinetes is a direct indicator of
258 fertilisation in the mosquito environment. In our study, we fed the same gametocyte material to the
259 three mosquito species. We reproducibly observed low transmission potential of the 3815 line, which
260 consistently formed very few ookinetes in all species and exhibited low and sporadic oocyst
261 infections. We also observed a tendency for African parasites to fare better in the African
262 mosquitoes, illustrated by both the numbers of mature ookinetes and oocysts densities. Although
263 our sample size is too small for a comprehensive analysis of the underlying biological mechanisms,
264 one could speculate that genes influencing the parasite's ability to evade the mosquito immune
265 system may play a role. Pfs47 is essential for *P. falciparum* infection in *An. gambiae* but not for *An.*
266 *stephensi* [48, 49]. Contrary to this, all three Southeast Asian parasite lines were able to infect African
267 mosquito vectors. Interestingly ARN1G achieved slightly higher ookinete numbers in *An. coluzzii* but
268 this did not translate into higher oocyst densities. Similar to our observations on sexual conversion
269 rates, we found no significant transmission advantage for ART-R parasite lines with reduced ART
270 susceptibility in the RSA.

271 To confirm the ability of the parasites to complete sporogony, we quantified sporozoite numbers in
272 mosquito salivary glands. Previous studies have suggested that K13 mutant lines might produce
273 larger oocysts [32], potentially indicating increased sporozoite production. While we did not observe
274 a clear increase in oocyst size (data not shown), sporozoite production varied between parasite lines.
275 The two Asian ART-R lines, each with a unique K13 mutation, exhibited significantly higher sporozoite
276 loads in at least one African mosquito species. This effect was most pronounced for ARN1G that

277 showed significantly higher sporozoite numbers in *An. coluzzii*, even after adjusting for oocyst density
278 within the same mosquito batch. Further studies are needed to confirm these findings and to explore
279 the underlying biological mechanisms. However, our results are consistent with the suggestion that
280 the K13 mutation may lead to larger oocysts [32], which could lead to increased sporozoite
281 production.

282 Lastly, we examined the impact of DHA exposure on transmission efficiency, hypothesising that
283 parasite lines with increased survival rate of asexual parasites under DHA exposure may show similar
284 survival advantages when gametocytes are exposed. Previous work has suggested that male
285 gametocytes with K13 mutations may have partial protection against DHA [32, 50]. Using *An. coluzzii,*
286 we tested two DHA concentrations and found no evidence that DHA was less effective against
287 mature gametocytes and subsequently reducing transmission in ART-R parasites. Immature
288 gametocytes are known to be more susceptible to artemisinins than mature gametocytes [32, 51].
289 Since we did not examine the impact of DHA on immature gametocytes, we cannot rule out
290 preferential survival of immature ART-R gametocytes. Even at the highest DHA concentrations, both
291 ART-S and ART-R African parasite lines occasionally infected mosquitoes. This observation is
292 consistent with previous *ex vivo* experiments [45] and aligns with clinical studies showing that
293 treatment with DHA-piperaquine fails to fully prevent transmission in the first weeks after treatment
294 [52].

295 In this study, we examined a limited number of ART-S and ART-R parasite lines across three mosquito
296 species. We observed no consistent evidence of increased gametocyte production or increased
297 mosquito infectivity. While the transmission-reducing effect of DHA was imperfect, its efficacy was
298 not reduced in gametocyte-producing lines with partial resistance to ARTs. To fully uncover the
299 discrete impacts of individual K13 mutations on gametocyte production, infectivity and gametocyte
300 resistance to ARTs, future studies using genetically engineered lines in controlled isogenic
301 backgrounds are required.

302

303 **Ethics declarations**

304 Experiments with *in vitro* cultured parasites and *Anopheles* mosquitoes at Radboud University
305 Medical Center were conducted following approval from the Radboud University Experimental
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488 # Tables

489 **Table 1. Parasite isolates**

Parasite Isolate	Origin	Drug resistance-conferring mutations				
		Artemisinin	Chloroquine	Mefloquine	Pyrimethamine	Sulfadoxine
NF54	Africa	-	-	-	-	-
NF180	East Africa (Uganda)	-	-	-	DHFR (N51I, S108N)	DHPS (A437G, K540E)
PAT-023	East Africa (Uganda)	-	PfMDR1 (Y184F)	-	DHFR (C59R, S108N)	DHPS (A437G, K540E)
NF135	Southeast Asia (Cambodia)	-	PfCRT (M74I, N75E, K76T, A220S, Q271E, I356T, R371I)	-	DHFR (N51I, C59R, S108N, I164L)	DHPS (S436A, A437G, K540E)
ARN1G	Southeast Asia (Thailand)	K13 (G449A)	PfCRT (M74I, N75E, K76T, A220S, Q271E, N326S, I356T, R371I)	-	DHFR (N51I, C59R, S108N, I164L)	DHPS (A437G, K540E, A581G)
3815	Southeast Asia (Cambodia)	K13 (C580Y)	PfCRT (M74I, N75E, K76T, A220S, Q271E, I356T, R371I)	-	DHFR (N51I, C59R, S108N, I164L)	DHPS (S436A, A437G, K540E)

490

491 ## Figure Legends

492 **Figure 1.** Parasite survival after exposure to dihydroartemisinin (DHA) and gametocyte conversion
493 rates of *P. falciparum* isolates with different K13 genotypes and genetic backgrounds. **A)** Survival rate
494 from the Ring-stage Survival Assay (RSA) of each parasite line following 6hr exposure to 700nM DHA
495 compared to DMSO controls in duplicate wells (4-6 replicates were performed for each parasite line).
496 The dots represent biological replicate survival rates at 72h post invasion from counts either by
497 microscopy or flow cytometry and the error bars represent the 95% confidence intervals. **B)** Sexual
498 conversion in a plate-based assay that quantifies conversion rates from a single asexual round using
499 three different media types (shown different colour gradient of the bars). Conversion rates are
500 calculated by dividing the final gametocytaemia by the starting parasitemia in the same well. Dots
501 represent a single well from independent plates; error bars represent 95% confidence intervals.

502 **Figure 2.** Infection of three mosquito species with the six different parasite lines. **A)** Ookinete counts
503 20 hours after blood meal. The ookinetes were stained with anti-Pfs25 488 conjugate and counted on
504 a haemocytometer under a fluorescent microscope. Immature forms included rounded zygotes (or
505 unfertilised females) and ookinetes with incomplete maturation (lighter shading bar), while mature
506 forms were only the completely mature ookinetes (dark shaded bar). Dots represent a single count
507 from an independent experiment and the error bars represent 95% confidence intervals. **B)** Average
508 oocysts per midgut were dissected on day 7 post-bloodmeal. A total of 20 mosquitoes per group
509 were dissected and oocysts were counted by mercurochrome staining. Dots represent the counted
510 oocysts from a single midgut; error bars represent 95% confidence intervals. **C)** Salivary glands from
511 individual mosquitoes were dissected and sporozoites quantified by qPCR. The dots represent the
512 sporozoites per salivary gland from a single mosquito and the error bars represent the confidence
513 intervals.

514 **Figure 3.** Transmission reduction in the presence of DHA. **A)** Oocyst densities observed upon
515 exposing mature gametocytes to 700nM and 7000nM DHA prior to being fed to mosquitoes in a
516 blood meal. The dots represent the average oocysts per infected mosquito from a single cage. **B)**
517 Relative reductions in oocysts density compared the no drug control. All error bars represent the
518 confidence intervals.

519 **Figure 4.** Impact of resistance on transmission and commitment. Transmission **A)** and conversion **B)**
520 plotted against survival rates from the RSA for each parasite line. The underlying detailed data on *in*
521 *vitro* parasite resistance in the RSA and gametocyte commitment are presented in Figure 1; data on
522 transmission under DHA exposure are presented in Figure 3.

524 ## Supplemental Tables

525 **Sup Table 1. RSA comparisons**

Reference parasite line	Parasite line	Ratio (CI)
NF54	NF180	0.99 (0.25, 3.85); p=0.9742
NF54	PAT-023	33.34 (10, 100); p<0.0001
NF54	NF135	6.67 (1.49, 33.34); p=0.0023
NF54	ARN1G	20.00 (6.25, 100); p<0.0001
NF54	3815	33.34 (12.5, 100); p<0.0001
NF135	PAT-023	4.31 (1.20, 15.49); p=0.0057
NF135	ARN1G	3.13 (0.81, 12.5); p=0.0636
NF135	3815	5.26 (1.51, 20); p=0.0014

526

527 **Sup Table 2. Sexual conversion comparisons**

Parasite line	Reference media	Media	Ratio (CI)
NF54	serum	mFa	114.14 (31.57, 412.62); p<0.0001
NF54	serum	Albumax	57.67 (15.77, 210.87); p<0.0001
NF54	mFa	Albumax	0.51 (0.16, 1.60); p=0.1516
NF180	serum	mFa	46.96 (14.23, 154.93); p<0.0001
NF180	serum	Albumax	21.99 (6.47, 74.73); p<0.0001
NF180	mFa	Albumax	0.47 (0.16, 1.39); p=0.0934
PAT-023	serum	mFa	3.95 (1.06, 14.70); p=0.0374
PAT-023	serum	Albumax	2.90 (0.78, 10.79); p=0.1027
PAT-023	mFa	Albumax	0.73 (0.20, 2.74); p=0.5691
NF135	serum	mFa	92.75 (25.51, 337.27); p<0.0001
NF135	serum	Albumax	27.16 (6.95, 106.13); p<0.0001
NF135	mFa	Albumax	0.29 (0.09, 1.01); p=0.0172
ARN1G	serum	mFa	75.74 (22.86, 250.88); p<0.0001
ARN1G	serum	Albumax	30.40 (8.75, 105.61); p<0.0001
ARN1G	mFa	Albumax	0.40 (0.13, 1.24); p=0.0511
3815	serum	mFa	7.27 (1.97, 26.84); p=0.0011
3815	serum	Albumax	4.30 (1.16, 16.00); p=0.0162
3815	mFa	Albumax	0.59 (0.16, 2.19); p=0.3311

528 mFa, minimal fatty acid.

529 **Sup Table 3. Oocyst comparisons**

Parasite line	Reference mosquito species	Mosquito species	Oocyst ratio
NF54	*An. stephensi*	*An. coluzzii*	4.35 (2.5, 7.69); p<0.0001
NF54	*An. stephensi*	*An. gambiae*	1.56 (0.87, 2.78); p=0.0695
NF54	*An. coluzzii*	*An. gambiae*	0.35 (0.20, 0.62); p<0.0001
NF180	*An. stephensi*	*An. coluzzii*	1.66 (1.01, 2.70); p=0.0285
NF180	*An. stephensi*	*An. gambiae*	2.17 (1.33, 3.57); p=0.0005
NF180	*An. coluzzii*	*An. gambiae*	1.31 (0.81, 2.12); p=0.1829
PAT-023	*An. stephensi*	*An. coluzzii*	6.25 (3.45, 11.11); p<0.0001
PAT-023	*An. stephensi*	*An. gambiae*	3.45 (1.96, 5.88); p<0.0001
PAT-023	*An. coluzzii*	*An. gambiae*	0.55 (0.32, 0.94); p=0.0079
NF135	*An. stephensi*	*An. coluzzii*	1.09 (0.66, 1.79); p=0.6706
NF135	*An. stephensi*	*An. gambiae*	0.69 (0.42, 1.12); p=0.1353
NF135	*An. coluzzii*	*An. gambiae*	0.63 (0.38, 1.03); p=0.0732
ARN1G	*An. stephensi*	*An. coluzzii*	0.53 (0.31, 0.9); p=0.0087
ARN1G	*An. stephensi*	*An. gambiae*	0.30 (0.18, 0.53); p<0.0001
ARN1G	*An. coluzzii*	*An. gambiae*	0.57 (0.32, 1.00); p=0.0167
3815	*An. stephensi*	*An. coluzzii*	0.44 (0.22, 0.9); p=0.0066
3815	*An. stephensi*	*An. gambiae*	0.08 (0.03, 0.24); p<0.0001
3815	*An. coluzzii*	*An. gambiae*	0.18 (0.06, 0.56); p=0.0006

530

531 **Sup Table 4. Sporozoite comparisons**

Parasite line	Reference mosquito species	Mosquito species	Ratio (CI)
NF54	*An. stephensi*	*An. coluzzii*	0.28 (0.04, 2.0); p=0.2362
NF54	*An. stephensi*	*An. gambiae*	0.11 (0.01, 0.79); p=0.0236
NF54	*An. coluzzii*	*An. gambiae*	0.38 (0.07, 2.02); p=0.2362
NF180	*An. stephensi*	*An. coluzzii*	1.30 (0.31, 5.26); p=0.6562
NF180	*An. stephensi*	*An. gambiae*	0.28 (0.07, 1.19); p=0.0702
NF180	*An. coluzzii*	*An. gambiae*	0.22 (0.05, 0.92); p=0.0333
PAT-023	*An. stephensi*	*An. coluzzii*	1.69 (0.17, 16.67); p=1.0000
PAT-023	*An. stephensi*	*An. gambiae*	1.59 (0.14, 16.67); p=1.0000
PAT-023	*An. coluzzii*	*An. gambiae*	0.93 (0.08, 10.51); p=1.0000
NF135	*An. stephensi*	*An. coluzzii*	0.09 (0.02, 0.46); p=0.0010
NF135	*An. stephensi*	*An. gambiae*	0.22 (0.04, 1.09); p=0.0488
NF135	*An. coluzzii*	*An. gambiae*	2.20 (0.48, 10.04); p=0.2114
ARN1G	*An. stephensi*	*An. coluzzii*	5.88 (1.35, 25.0); p=0.0084
ARN1G	*An. stephensi*	*An. gambiae*	0.35 (0.06, 1.92); p=0.1388
ARN1G	*An. coluzzii*	*An. gambiae*	0.06 (0.01, 0.29); p<0.0001
3815	*An. stephensi*	*An. coluzzii*	0.02 (0, 0.31); p=0.0011
3815	*An. stephensi*	*An. gambiae*	2.56 (0.2, 33.34); p=0.3758
3815	*An. coluzzii*	*An. gambiae*	106.97 (8.32, 1375.20); p<0.0001

532

533

534 **Sup Table 5. Sporozoite per oocyst comparisons**

Parasite line	Reference mosquito species	Mosquito species	Sporozoite per oocyst ratio
NF54	*An. stephensi*	*An. coluzzii*	0.06 (0.01, 0.50); p=0.0044
NF54	*An. stephensi*	*An. gambiae*	0.07 (0.01, 0.56); p=0.0047
NF54	*An. gambiae*	*An. coluzzii*	0.92 (0.16, 5.33); p=0.9092
NF180	*An. stephensi*	*An. coluzzii*	0.78 (0.17, 3.51); p=0.6919
NF180	*An. stephensi*	*An. gambiae*	0.13 (0.03, 0.60); p=0.0042
NF180	*An. gambiae*	*An. coluzzii*	5.89 (1.32, 26.36); p=0.0096
PAT-023	*An. stephensi*	*An. coluzzii*	0.27 (0.03, 2.88); p=0.5525
PAT-023	*An. stephensi*	*An. gambiae*	0.46 (0.04, 5.59); p=0.9104
PAT-023	*An. gambiae*	*An. coluzzii*	0.59 (0.05, 7.06); p=0.9104
NF135	*An. stephensi*	*An. coluzzii*	0.09 (0.02, 0.45); p=0.0012
NF135	*An. stephensi*	*An. gambiae*	0.32 (0.06, 1.74); p=0.1198
NF135	*An. gambiae*	*An. coluzzii*	0.29 (0.06, 1.42); p=0.1198
ARN1G	*An. stephensi*	*An. coluzzii*	11.30 (2.31, 55.28); p=0.0009
ARN1G	*An. stephensi*	*An. gambiae*	1.15 (0.19, 6.97); p=0.8499
ARN1G	*An. gambiae*	*An. coluzzii*	9.81 (1.79, 53.69); p=0.0028
3815	*An. stephensi*	*An. coluzzii*	0.05 (0.00, 0.76); p=0.0084
3815	*An. stephensi*	*An. gambiae*	31.40 (1.95, 504.25); p=0.0062
3815	*An. gambiae*	*An. coluzzii*	0.00 (0.00, 0.03); p<0.0001

535

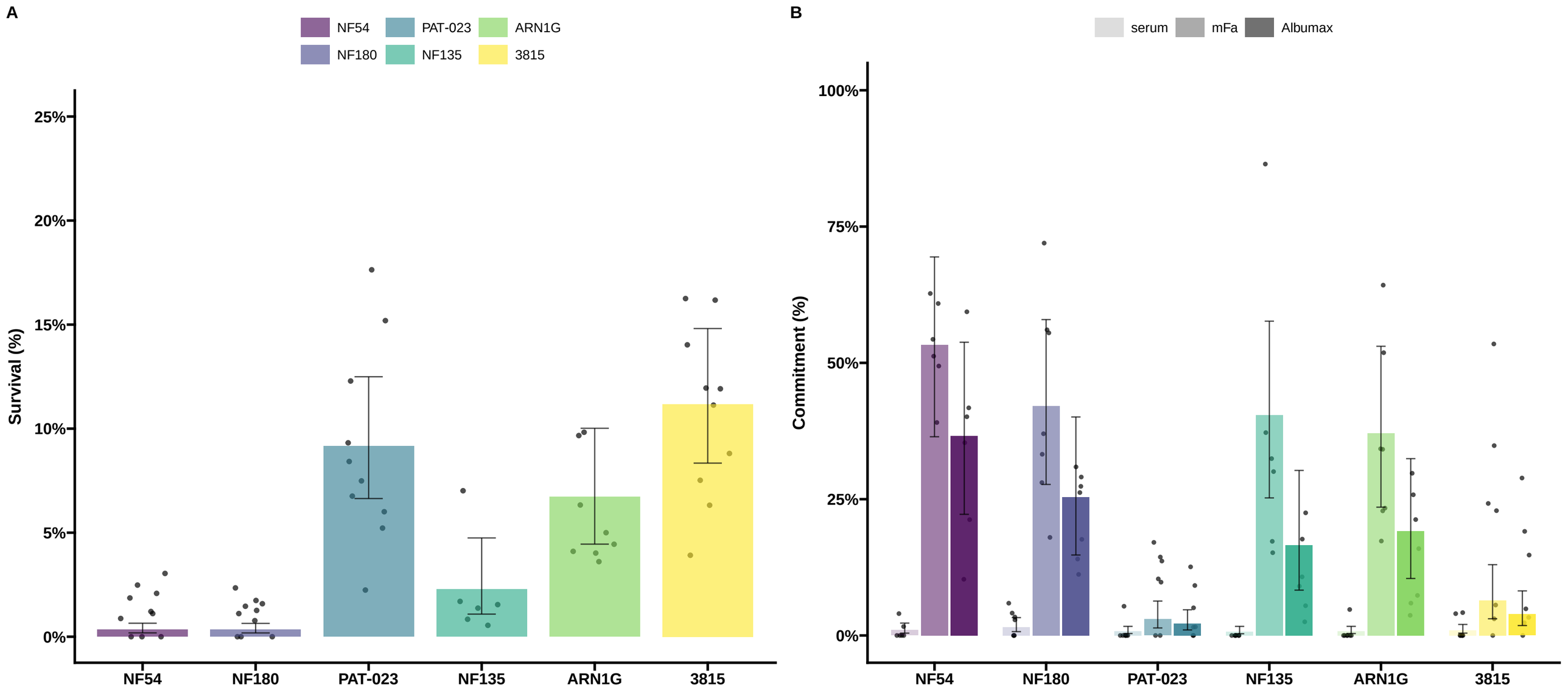
536 ## Supplemental Figure Legends

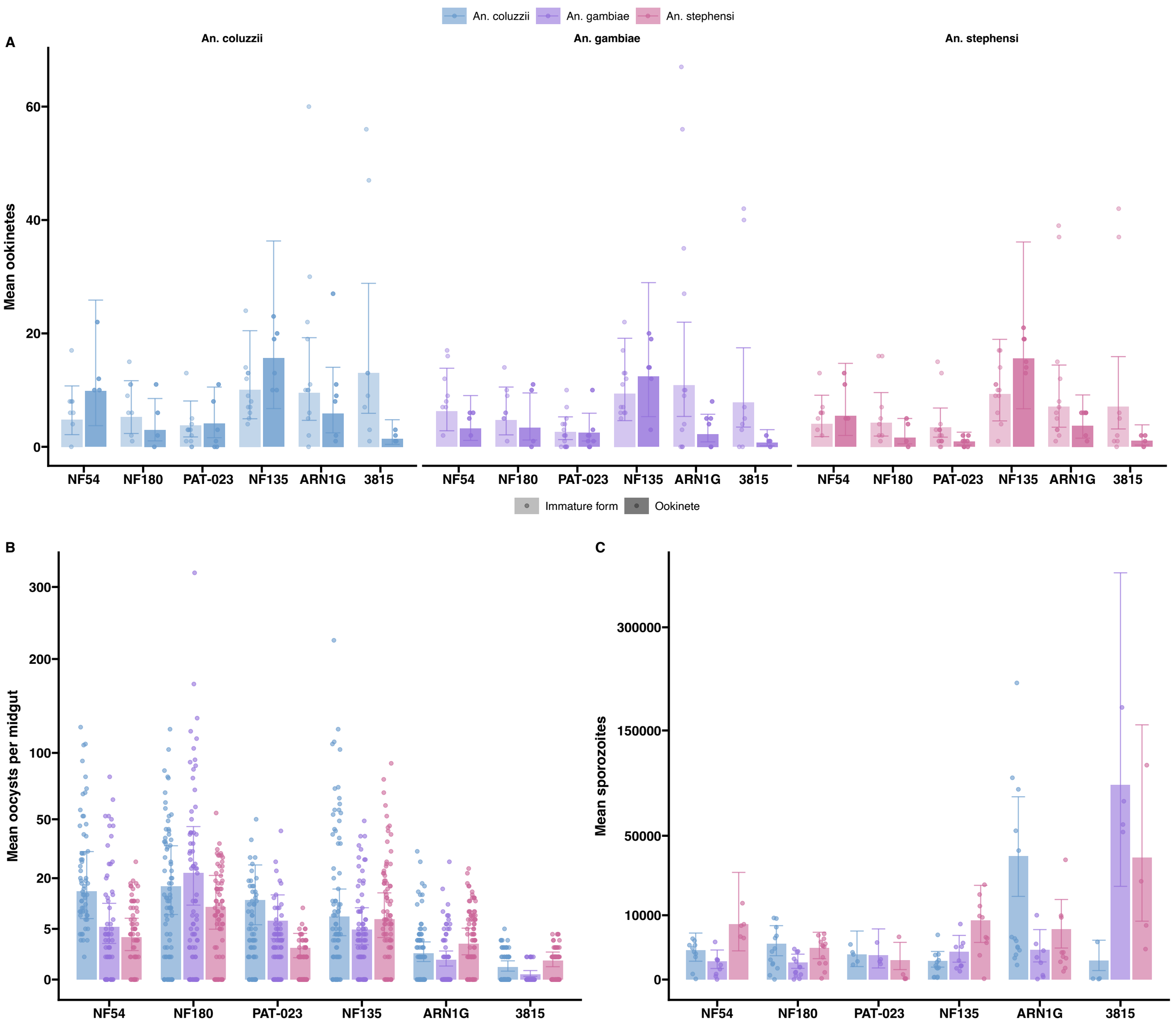
537 **Sup Figure 1.** Mosquito infection rates for the six parasite lines in each mosquito species across three
538 to four independent mosquito feeding experiments.

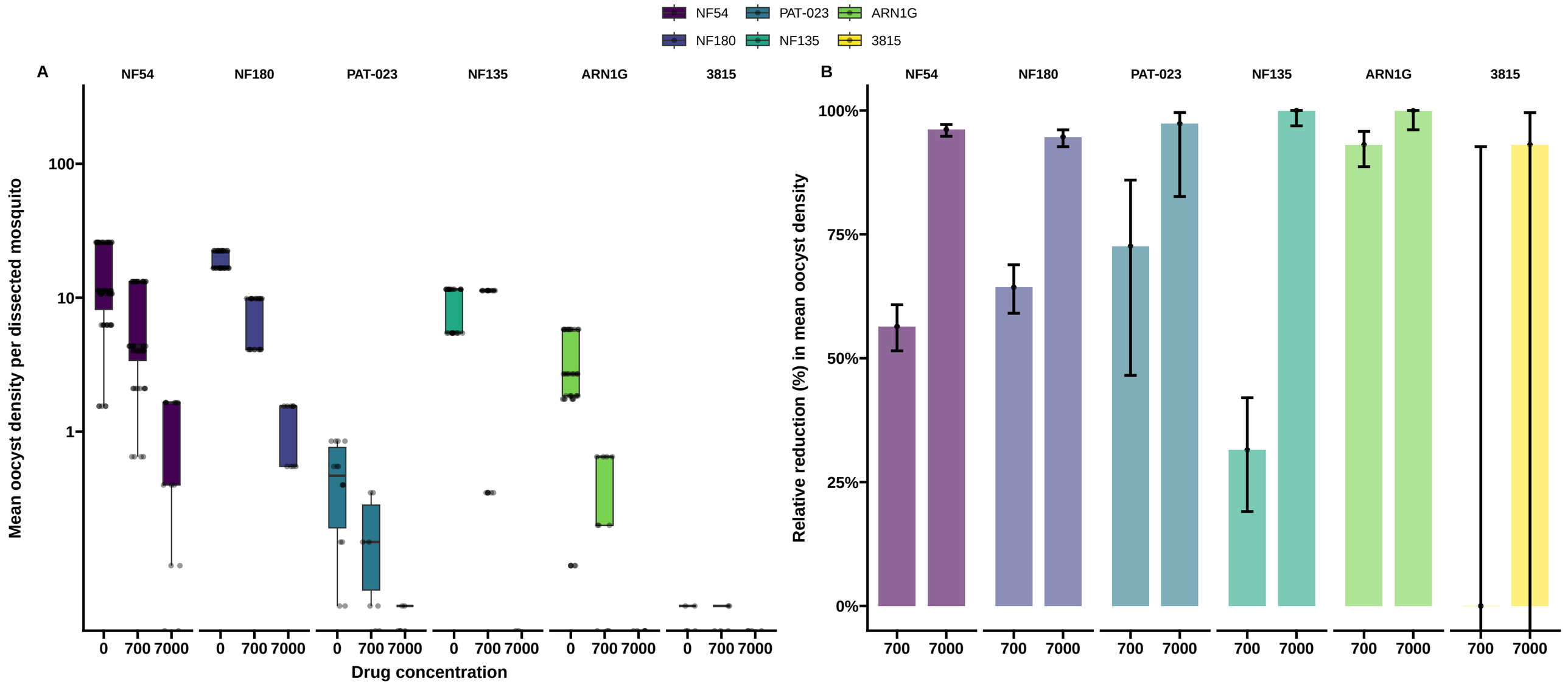
539 **Sup Figure 2.** Calculated sporozoite per oocyst**.** Sporozoites per salivary gland from individual
540 mosquitoes were dissected and processed for qPCR. The sporozoites per oocysts was calculated
541 based on the cage matched oocysts per mosquito average. The error bars represent the confidence
542 intervals.

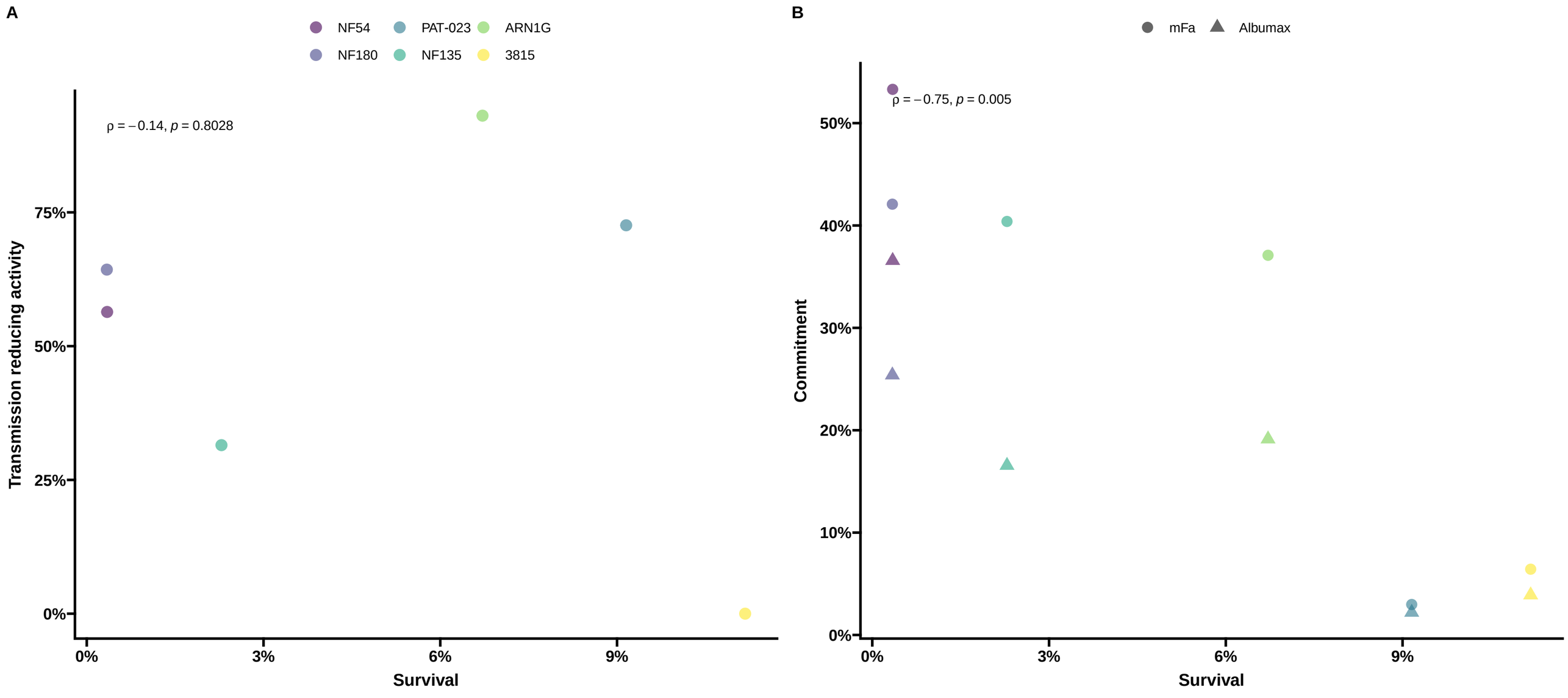
543 **Sup Figure 3.** Reduction in the overall oocyst prevalence of the six parasites lines following exposure
544 to DHA. **A)** Shows the decrease in mosquito infectivity upon exposing mature gametocytes to 700nM
545 and 7000nM DHA prior to being fed to mosquitoes in a blood meal. The dots represent the
546 prevalence from an individual mosquito cage. **B)** Represents the relative reduction in oocysts density
547 compared the no drug control. All error bars represent the confidence intervals.

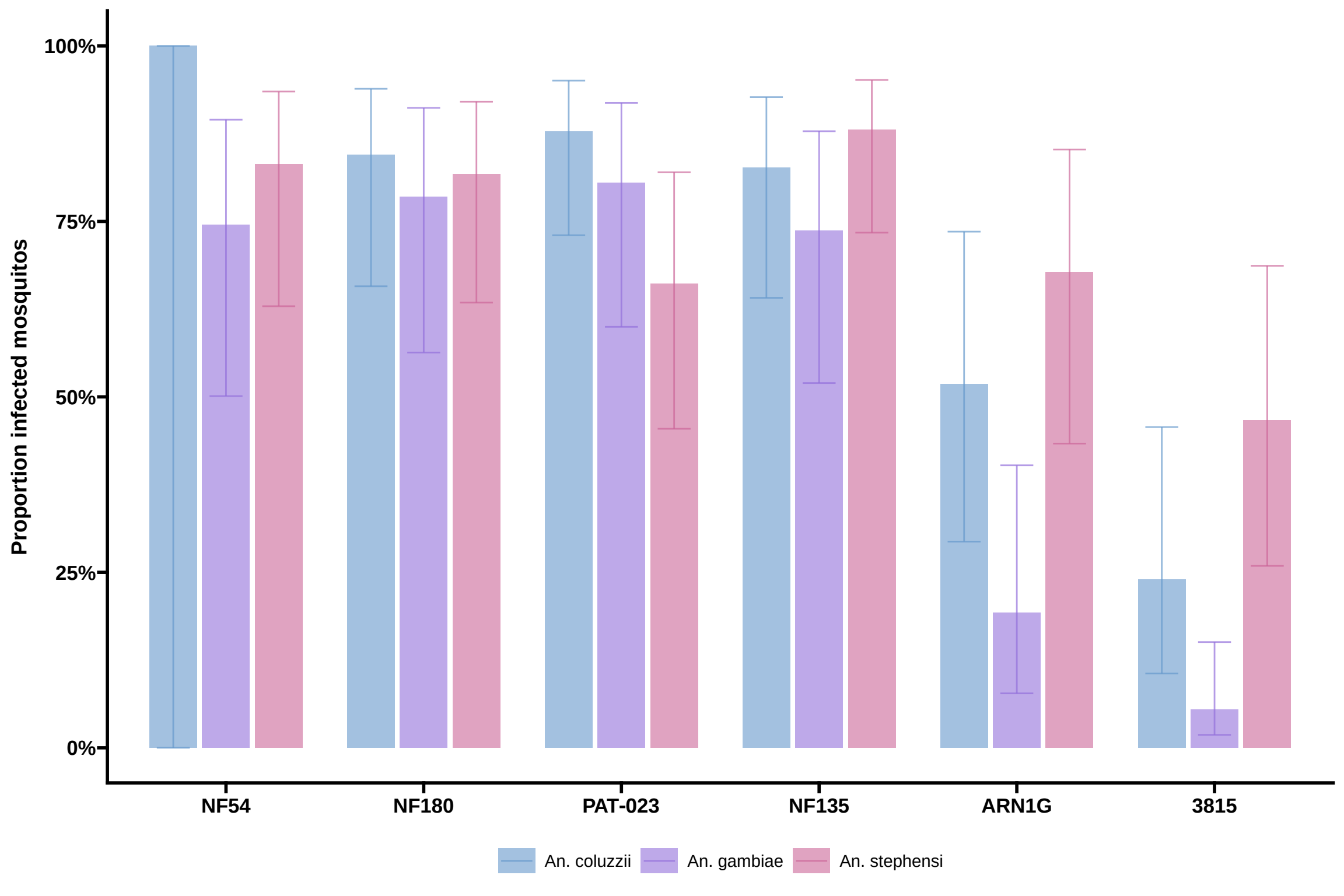
548

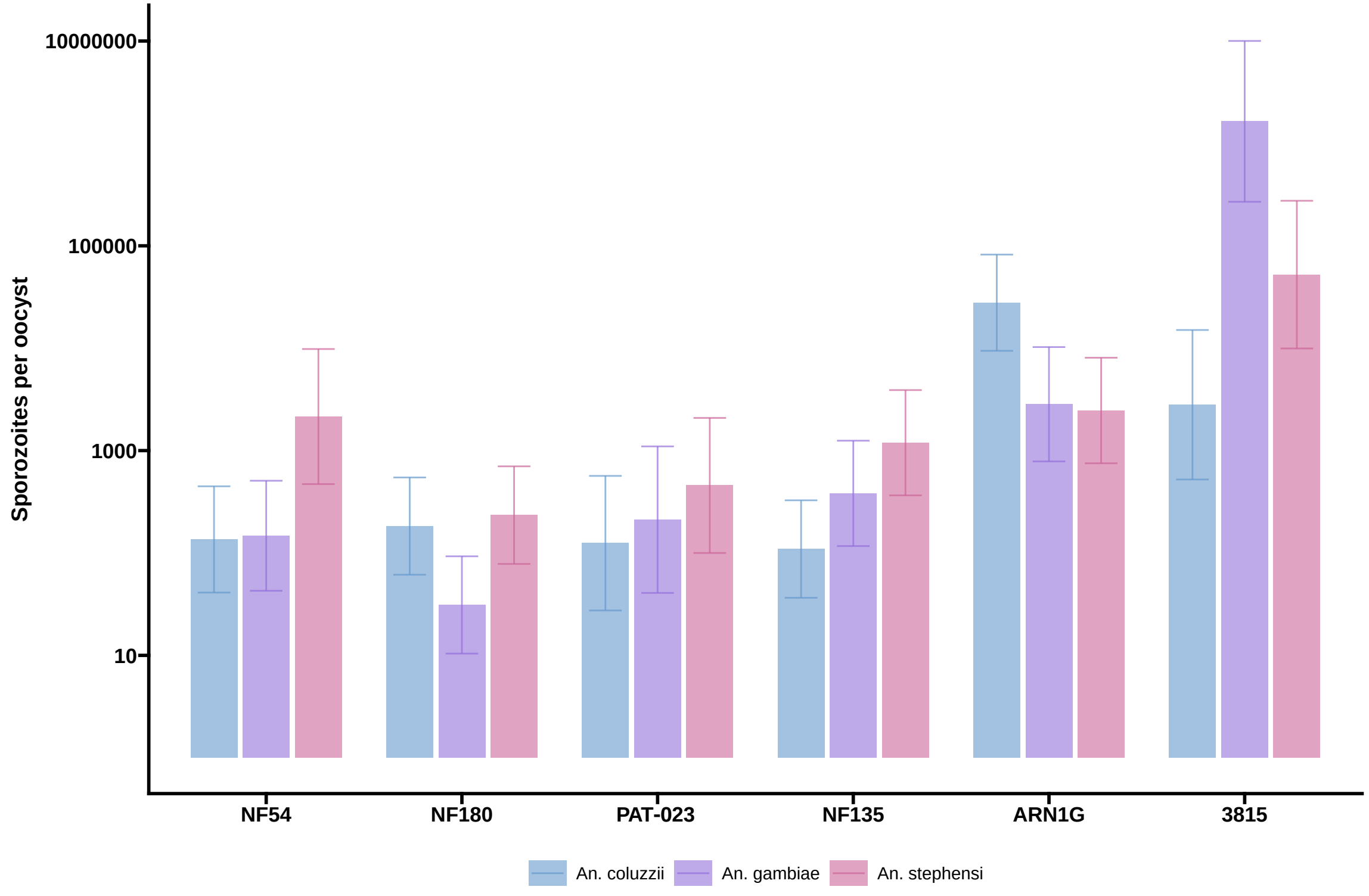

A
NF54
PAT-023
ARN1G
NF180
NF135
3815
Survival (%)
25%
20%
15%
10%
5%
0%
NF54
NF180
PAT-023
NF135
ARN1G
3815
B
serum
mFa
Albumax
Commitment (%)
100%
75%
50%
25%
0%
NF54
NF180
PAT-023
NF135
ARN1G
3815

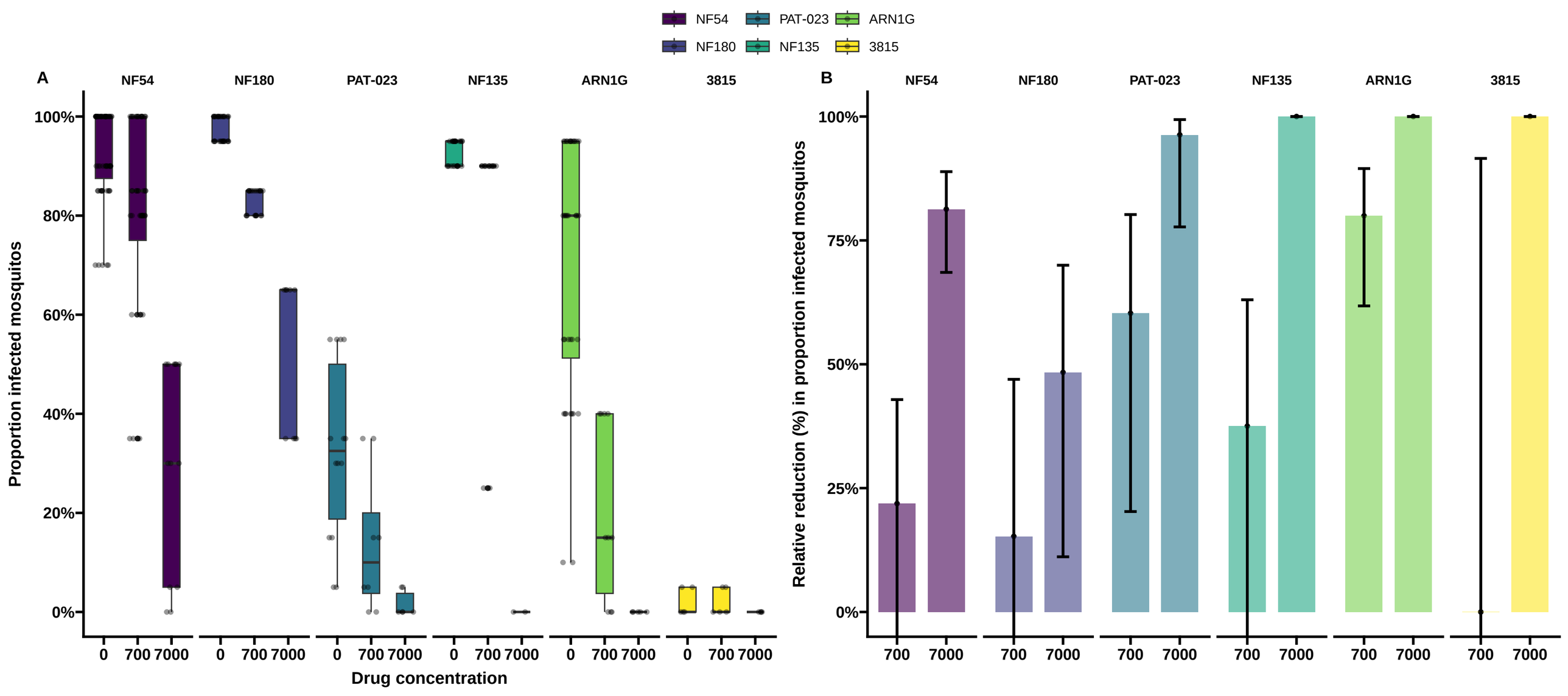

An. coluzzii
An. gambiae
An. stephensi
A
Mean ookinetes
0
20
40
60
NF54
NF180
PAT-023
NF135
ARN1G
3815
Immature form
Ookinete
B
Mean oocysts per midgut
0
5
20
50
100
200
300
C
Mean sporozoites
0
10000
50000
150000
300000


NF54
PAT-023
ARN1G
NF180
NF135
3815
A
NF54
NF180
PAT-023
NF135
ARN1G
3815
Mean oocyst density per dissected mosquito
100
10
1
0
700
7000
Drug concentration
B
NF54
NF180
PAT-023
NF135
ARN1G
3815
Relative reduction (%) in mean oocyst density
100%
75%
50%
25%
0%
700
7000


A
NF54
PAT-023
ARN1G
NF180
NF135
3815
ρ = −0.14, p = 0.8028
Transmission reducing activity
75%
50%
25%
0%
0%
3%
6%
9%
Survival
B
mFa
Albumax
ρ = −0.75, p = 0.005
Commitment
50%
40%
30%
20%
10%
0%
0%
3%
6%
9%
Survival


Proportion infected mosquitos
100%
75%
50%
25%
0%
NF54
NF180
PAT-023
NF135
ARN1G
3815
An. coluzzii
An. gambiae
An. stephensi


Sporozoites per oocyst
10000000
100000
1000
10
NF54
NF180
PAT-023
NF135
ARN1G
3815
An. coluzzii
An. gambiae
An. stephensi


NF54
PAT-023
ARN1G
NF180
NF135
3815
A
B
Proportion infected mosquitos
Relative reduction (%) in proportion infected mosquitos
Drug concentration
100%
80%
60%
40%
20%
0%
75%
50%
25%
0
700
7000