1 # Differential effects of multiplex and uniplex affiliative relationships on

2 # biomarkers of inflammation

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Abstract

23

24   Social relationships profoundly impact health in social species. Much of what we know

25   regarding the impact of affiliative social relationships on health in nonhuman primates (NHPs)

26   has focused on the structure of connections or the quality of relationships. The influence of

27   relationship dimensionality (e.g., the breadth of affiliative behaviors used) on health and fitness

28   outcomes remains unknown. Here we explored how social networks containing dyads with either

29   multiplex (dyads both groom and huddle) or uniplex (dyads only groom) affiliative relationships

30   differ in their structure and association with biomarkers of inflammation, an indicator of

31   individual health risk. Being strongly embedded in multiplex affiliative networks, which were

32   more modular and kin biased, was associated with lower inflammation (IL-6, TNF-alpha). In

33   contrast, being well connected in uniplex networks, which were more strongly linked with social

34   status, was associated with greater inflammation. Results suggest that multiplex affiliative

35   relationships may function as supportive relationships that promote health. In contrast, the

36   function of uniplex affiliative relationships may be more transactional and may incur

37   physiological costs. This complexity is important to consider for understanding the mechanisms

38   underlying the association of social relationships on human and animal health.

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40   Keywords: affiliation, *Macaca mulatta*, cytokines, inflammation

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Introduction

For decades, research has shown that social relationships impact individual health and fitness with estimates of the magnitude of the association with mortality on par with other well recognized mortality risks (e.g., smoking, alcohol consumption, obesity)[1]. Researchers often distinguish between structural (quantity and structure) versus functional (quality or function) measures of social relationships, and while only moderately correlated (r = 0.2-0.3) both high quantity and quality of relationships have been associated with better health outcomes[1,2]. Notably, multidimensional measures (i.e., those that assess multiple aspects of social connections) often are the strongest predictors of health and fitness[1–3]. While structural measures of social relationships (number of social partners) are typically straightforward for both human and animal societies, functional measures of social relationships are more feasible and thus common for humans (e.g., measures of perceived social support), whereas determining the function of social relationships in animals is much more complicated.

Quantitative measures of the structure of affiliative social relationships are often simple to calculate and include the number of social partners, the frequency of interactions, or the higher order structuring of social relationships using social network analysis. While some studies find that the total number of social connections or the total amount of affiliation are associated with survival and reproductive outcomes, others find that only *certain* relationships (e.g., animals: strong and stable social bonds; humans: family and friends) impact health and fitness[1,2,4–9]. Social network analysis is often used to examine how structural features of social life or an individual's role in their broader social network or community may impact their health and wellbeing. Commonly used network centrality metrics include eigenvector, betweenness, and closeness which reflect the degree to which an individual has social capital, acts as a bridge

65   between others, and is embedded in the network, respectively. Less commonly investigated is

66   the impact of clustering or transitivity (the degree to which your connections are connected to

67   each other) a measure of subgrouping and the degree to which individual form cohesive

68   "cliques"[10]. While the general pattern seen across these measures is that greater connectedness

69   or centrality is associated with lower risk for gastrointestinal pathogens, increased reproduction

70   and longevity[4,10–12], there is little consistency across studies in identifying which specific social

71   role or network metric is important and some studies find no impact of social network position

72   on fitness[2,4,6].

73   Measuring the function of social relationships attempts to examine what purpose or role a

74   relationship serves and whether it meets the needs of the individual. In humans, functional

75   measures of social relationships include surveys of perceived social support, informational

76   support, emotional support, and tangible support. Assessing functions of different relationships

77   is far more challenging in animals due to the fact we cannot ask animals the value or perceptions

78   of their relationships. Instead, research on social relationships in animals has more often relied

79   on metrics designed to indirectly assess the quality rather than directly query the function of

80   relationships. A commonly used metric to assess the quality of affiliative social relationships is

81   the dyadic sociality index (DSI) which incorporates information from multiple, correlated

82   affiliative behaviors (e.g., grooming and proximity). Relationships with high DSI scores are

83   commonly referred to as strong bonds and tend to be equitable, stable, involve frequent

84   interaction and are most common between kin and peers[4,13,14]. Higher number or quality of these

85   strong bonds has been associated with acute hormonal responses (e.g., oxytocin or cortisol

86   levels), increased reproduction, and survival[4,6,7,15]. While the positive association between strong

87   bonds and increased reproduction and survival are well supported (although see[16]), the strength

88   of affiliative bonds is often skewed with strong affiliative bonds making up only a small fraction

89   of affiliative relationships an individual has[7,13,17–19]. The function of these other, weaker bonds

90   has been hypothesized to be increased social flexibility (e.g., social connections can shift with

91   environmental demands), with general social integration and indirect connections providing

92   access to others that may have resources or information[4,17]. However, evidence for an

93   association between weak bonds and health and fitness is mixed[7,17]. While useful, the dichotomy

94   of strong and weak bonds does not fully characterize these relationships and their potential

95   functions. Hinde[20] points out that "affection is multidimensional and cannot be assessed along a

96   simple scale…" (p. 13). We know that both structural and functional aspects of social

97   relationships are key to understanding their impacts on health in humans. Therefore, to better

98   understand the mechanisms by which these relationships impact health and fitness in animals we

99   need additional tools to characterize social relationships.

100   Recent advances in social network analysis and theory, and specifically multiplex

101   networks, may provide tools to help disentangle the impact of structural and functional social

102   relationships. Most studies of animal affiliation either analyze behaviors separately (e.g.,

103   grooming or proximity) or lump affiliative behaviors together giving them roughly equal

104   weight[4,21]. However, such practices may overlook key information in the diversity or breadth of

105   affiliative interactions in which a dyad engages [20,21]. Evidence for the importance of

106   multidimensionality in social relationships can be found in a study by Balasubramaniam and

107   colleagues[11] in which they found that highly connected rhesus macaques (i.e., high outdegree or

108   eigenvector) in a grooming network were less likely to have *Shigella*, a gastrointestinal

109   pathogen, but only if they were also well connected in a huddling network (i.e., high

110   betweenness). This finding lends support to the idea that the breadth or diversity of affiliative

111   behaviors a dyad engages in might provide important information as to the nature of that

112   relationship and its potential impacts on health and fitness[11]. Therefore to understand the impact

113   of social relationships on individual health and fitness, we must examine the cumulative impact

114   of an individual's diversity and breadth of social relationships to really understand their potential

115   influence on health[22,23]. Social network approaches provide an important tool to aid in bridging

116   the gap between these different levels of analysis: from interactions, to relationships, to an

117   individual's place in a social structure and may be uniquely suited to assessing the cumulative

118   impact of social relationships on individual functioning.

119   Here we used social network analysis to explore whether behavioral diversity within a

120   relationship can allow for understanding of the potential function of those relationships and their

121   impact on health-related outcomes. Affiliation in primates takes many forms, including

122   grooming, huddling, proximity, embracing, and less commonly coalitionary support[43–45]. In

123   macaques, grooming is commonly used to indicate the presence of an affiliative relationship[41,46].

124   Grooming has been proposed to serve multiple social functions including: to maintain social

125   bonds[46] and social cohesion[47], and in exchange for tolerance from dominants, for agonistic

126   support, or for access to resources[48–50]. Although less commonly studied, huddling behavior may

127   also be an important indicator of strong affiliative relationships[51], particularly those that may

128   offer social buffering[11]. Therefore, we generated networks consisting of dyads with multiplex

129   (i.e., dyads both groomed and huddled) vs. uniplex (i.e., dyads that only groomed) affiliative

130   relationships and examined whether they differed in their network structure in ways that might

131   allow us to infer their potential function (e.g., social bonding and cohesion or exchange for

132   tolerance and resources). The structure of the resulting networks was then examined in relation

133   to key structural features of rhesus relationships including: modularity (i.e., degree of clustering),

134 associations with kinship, and associations with rank[46,47]. Next, we determined whether the

135 structure of connections in multiplex and uniplex affiliative networks were associated with

136 biomarkers of inflammation (i.e., serum pro-inflammatory cytokines), an indicator of individual

137 health status. Chronic inflammation is associated with for a variety of diseases (e.g., diabetes,

138 cardiovascular disease, cancer) and mortality[24–26]. We measured two pro-inflammatory

139 cytokines, IL-6 and TNF-α, as biomarkers of general inflammation. High levels of these pro-

140 inflammatory cytokines and have previously been reported to be associated with social variables

141 (e.g., low social status, low social integration, poor quality relationships, loneliness) in humans

142 and rhesus macaques[22,27–30].

Materials and Methods

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Subjects and housing

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145 Subjects were 248 breeding age (3 years and older) female rhesus macaques (*Macaca*

146 *mulatta*) that were born at the California National Primate Research Center in Davis, California

147 (Table 1). Subjects lived in one of four large multigenerational and matrilineal social groups

148 containing 100-200 mixed-sex individuals (Table 1), each housed in a 0.2 hectare outdoor

149 enclosure. Subjects were fed commercial monkey chow and foraging enrichment twice daily.

150 Fruits or vegetables were provided weekly. Water was available ad libitum.

151 **Table 1: Group Demographics**

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Group	Group size (adults)	N (adult females)	# of Matrilines[a]	Mean Matriline[a] size (SD)
Group A	131 (101)	74	13	5.7 (3.6)
Group B	204 (101)	67	33	2.0 (1.0)
Group C	125 (55)	39	6	6.5 (3.9)
Group D	185 (96)	68	13	5.2 (2.3)

160 [a] Number of matrilines and matriline size statistics include only breeding age females.
161 Individuals were considered part of the same matriline if they could be traced back to the same
162 female genetic common ancestor at the time of group formation.

163

164   Rhesus macaques live in large multi-male, multi-female social groups organized by rank

165   and kinship[31]. For females, rank is inherited from their mothers and generally all members of a

166   matriline hold adjacent ranks[32] (although see[33]). In contrast, males generally immigrate into a

167   new social group and may enter at the bottom of the hierarchy, queueing for rank, or attain rank

168   through direct competition[34]. Rhesus macaque females form the core of the social group with

169   affiliation between both kin and non-kin playing a key role in maintaining group stability[31,35].

170   Although male social bonds have important fitness outcomes in macaques generally[36], male

171   rhesus macaques engage in social affiliation far less frequently[37] and tend to be more socially

172   isolated than females[38]. Therefore, we focus our analysis on females, which we predict will be

173   more strongly impacted by social bonds than males. We use rhesus macaques as a group-living,

174   nonhuman primate (NHP) model because their social relationships are highly differentiated,

175   exhibit a high degree of complexity and individual variability, and have been linked to a variety

176   of health and fitness outcomes[22,30,39,40].

177   **Behavioral data collection**

178   Subjects were part of a larger study on the associations between social networks and

179   health. Groups A and B were studied for six continuous weeks during the birthing season from

180   March to April 2013 and 2014, respectively. Groups C and D were studied for six continuous

181   weeks during the breeding season from September to October 2013 and 2014, respectively.

182   Behavioral data were collected six hours per day, four days per week from 0900-1200 and 1300-

183   1600 each day by one of three observers (inter-rater reliability, Krippendorff's alpha ≥0.85).

184   Affiliative behavior was collected via scan sampling every 20-minutes (maximum 18 scans per

185   day), where identities of all adult female dyads affiliating (i.e. grooming or huddling) were

186   recorded[11]. Grooming was defined as cleaning or manipulating the fur of another animal and

187   huddling included all forms of body contact, including (but not restricted to) ventral contact,

188   embrace, or side by side sitting. During each scan, these behaviors were mutually exclusive for a

189   dyad (an individual grooming another was not also huddling that individual). Affiliation scans

190   produced 1637 scans (Group A: N=418, Group B: N=410, Group C: N=378, Group D: N=431)

191   and an average of 20.1 grooming interactions per female (group range 12.8 – 25.6) and 15.65

192   huddling interactions (group range 7.8-26.9). This sampling scheme has been shown to produce

193   sufficiently sampled grooming and huddling networks[41]. Aggression data (threats, chases, bites)

194   were also collected via an event sampling protocol for six hours per day, four days per week by

195   two other observers (average of 42.5 interactions per individual, group range 36.2 – 51.9).

196   Because social status has been shown to impact inflammation[39] (although see[22]), dyadic

197   aggression data was used to calculate dominance ranks and dominance certainty via the R

198   package *Perc*[22,42]. Dominance rank was expressed as the percent of animals in the group

199   outranked and therefore ranged from 0 (low) to 1 (high).

200   **Affiliative network analysis**

201   Huddling behavior was used to filter edges in the grooming network into two affiliative

202   networks; a network containing edges for dyads that both groomed and huddled at some point

203   during the 6-week study (multiplex affiliative relationships) and a network containing edges for

204   dyads that groomed but were never seen huddling (uniplex affiliative relationships). Edge-

205   weights in both networks reflected only the number of times that dyad was observed grooming

206   (Table S1). For each network, centrality and cohesion measures for each individual were

207   calculated in Cytoscape 3.7.1 using two plug-ins, NetworkAnalyzer and CytoNCA[52,53]. For all

208   metrics, both unweighted and weighted metrics were evaluated; unweighted metrics evaluated

209   the importance of who individuals were connected to regardless of how often they interacted

210   while weighted metrics evaluated whether the frequency with which animals interacted was

211   relevant. The effects of the direct connections for individuals were measured using degree

212   centrality. The effect of an individual's indirect connections in the network was evaluated using

213   eigenvector, betweenness, information, and closeness centralities[4,10,11]. In addition, the degree to

214   which individuals were part of cohesive local communities was measured by the local clustering

215   coefficient (i.e., triadic closure). Multiple metrics were chosen to reflect the different ways social

216   integration can manifest (e.g., bridging, cohesion, embeddedness, etc. Table 2).

217   **Table 2: Network Metric Definitions**
218   [a] Both weighted and unweighted metrics were used.

Measure	Description
Degree	measures the *number* (unweighted) of partners or *frequency* of interaction (weighted) for each node.
Eigenvector [a]	measures whether individuals are well connected to others that are also well connected, a measure of *social capital*.
Betweenness [a]	measures the number of times a node lies on the shortest path between other nodes, which reflects an individual's role in connecting others in the network or acting as a *social bridge*.
Information[a]	measures the number of times a node lies on *any* path between other nodes, which reflects an individual's role as a social bridge between many others. Reflects an individual's role in *social cohesion*.
Closeness[a]	measures how close each node is to all other nodes within the network, which reflects how *embedded* an individual is in the network
Clustering Coefficient	measures the extent to which a node's neighbors are also connected to each other, a measure of *cliquishness*.

219

Biological sample collection

221   Blood samples were taken during the fifth week of each group's study period during

222   routine, semi-annual health checks. On a single morning, all animals in a group were lightly

223   sedated with ketamine (10 mg/kg) and given veterinary exams. Blood samples were obtained

224   from the femoral vein and serum was aliquoted and stored at −80 °C for later assay. The order in

225   which animals were processed and samples were collected was recorded to control for any

226   potential impacts of the sampling procedure on the physiological variables examined.

Pro-inflammatory Cytokine Assay

Serum levels of IL-6 and TNF-α were measured simultaneously using commercially available, species specific Milliplex multi-analyte profiling (MAP) reagents purchased from EMD/Millipore (Billerica, MA, USA), and utilizing Luminex Xmap technology (Luminex, Austin, TX, USA). Color coded polystyrene microbeads coated with specific antibodies for IL-6 and TNF-α were incubated with the serum samples, washed, and then further reacted with biotinylated detector antibodies followed by Streptavidin-PE to label the immune complexes on the beads. After a final washing to remove all unbound material, the beads were interrogated in a BioPlex dual laser (BioRad, Hercules, CA, USA). The median fluorescent index for each sample was compared to a standard curve to calculate the concentration (IL-6: mean = 12.55 pg/mL, sd = 46.92, range = 0 – 690; TNF-α: mean = 185.0 pg/mL, sd = 442.27, range = 0 – 4052; see Figure S2 for histograms). Samples were tested in duplicate and had an intra-assay coefficient of variability of 15.3%. Samples were re-analyzed if the CV was greater than 25% for all analytes measured. Manufacturer provided quality control samples fell within recommended ranges for all assays. Samples falling below the threshold sensitivity of the assay (1.6 pg/mL) were assigned a value of zero (IL-6: N = 76, TNF-α: N = 53).

Statistical analysis

Two sets of analyses were done to determine whether 1) multiplex and uniplex affiliation networks differ in structure and relationships to known social features of rhesus macaques (e.g., kin bias, hierarchical organization), and 2) whether network metrics from these two networks predicted biomarkers of inflammation.

First, we compared multiplex and uniplex affiliation networks, which were treated as weighted and directed networks, to determine if they exhibited differences in key structural

250   features of rhesus relationships. For example, evidence suggests that despotic macaques such as

251   rhesus, particularly in large groups, are likely to have grooming networks that are modular (i.e.,

252   shows subgrouping), expected to be based on kinship, and have individual network positions

253   (i.e., eigenvector centrality) that are correlated with rank[55,56]. Therefore, we examined whether

254   these two networks differed in the degree of clustering (Newman's modularity, clustering

255   coefficient), kin bias (e.g., proportion of kin (kin unweighted degree/total unweighted degree)),

256   and associations with rank (proportion of grooming up the hierarchy, rank disparity among

257   grooming dyads) for each of the four groups studied. Also, because previous research has

258   focused on bond strength, we further examined reciprocity, strength of relationships (average

259   edge weight), and distribution of grooming (eigenvector centralization) across these network

260   types. Due to the low number of groups in the comparison, paired t-tests were used to evaluate if

261   the multidimensional vs unidimensional network metrics were consistently different across

262   groups. Normality of the differences was evaluated using the Shapiro-Wilk test, and if

263   significant then Wilcoxon signed rank tests were used. As a final structural analysis, we

264   examined the correlations between individual level network positions from these two network

265   types (Table S2) to evaluate multicollinearity within networks and associations between

266   networks.

267   Next, to determine if the structure of individual affiliative relationships in multiplex or

268   uniplex affiliation networks was associated with pro-inflammatory cytokines we ran generalized

269   linear models using a negative binomial distribution (proc glimmix, SAS 9.4) on each biomarker

270   separately (see [22] for details on distribution choice and Figure S2 for distributions). For these

271   analyses networks were treated as weighted but undirected. Model building proceeded in four

272   steps for each outcome (i.e., IL-6, TNF-α). One animal was excluded from the IL-6 analysis

273   because it was an outlier with influence (Cook's D >1); all other outliers had a Cook's D < 0.5

274   and therefore were included in the analyses.   A second animal was excluded from all analysis

275   due to the fact she was not included in the uniplex network. For all steps, ΔAIC > 2 was used to

276   identify potential predictors and candidate models. First, a random effect indicating the group ID

277   was evaluated for each outcome, and all subsequent models were compared to this random

278   effects only model. Next, variables from the literature (age, dominance rank, dominance

279   certainty, sampling order), although not of direct interest here, were evaluated to determine if it

280   was necessary to control for their effects on inflammation before examining social network

281   variables.   Third, a statistical winnowing strategy was used to eliminate nonpredictive social

282   network variables (multiplex affiliation, uniplex affiliation, or huddling metrics) from further

283   consideration[56]. Because huddling behavior was used to separate relationships into the multiplex

284   and uniplex networks, huddling degree was also included as a potential predictor to evaluate if it

285   was multiplex vs uniplex relationships or just simply huddling behavior driving observed effects.

286   While some multiplex affiliation and huddling metrics were highly correlated (Table S2), VIF

287   (range: 1.93 – 1.94) and tolerance (range: 0.517 – 0.519) did not indicate multicollinearity. A

288   final set of models was constructed in which variables identified in step 3 were combined to

289   identify a final set of candidate models. Metrics from the same network were never included in

290   the same model due to the interdependence of network metrics. If no single best model emerged,

291   candidate models (i.e., those with ΔAIC ≤ 2, Table S3) are discussed with model weights guiding

292   our interpretations[57]. A log of all models tested is available in Tables S3-5.

293   **Ethical Note**

294   All procedures used in this study met all legal requirements of the United States as well

295   as guidelines set by the American Society of Primatologists regarding the ethical treatment of

296   non-human primates. This study was approved by the Institutional Care and Use Committee at

297   the University of California, Davis and was carried out in compliance with the ARRIVE

298   guidelines.

299 Results

300   *Multiplex vs. Uniplex Affiliation Networks*

301   For all groups studied, clear differences in network topology, kinship, and associations

302   with dominance rank were seen between the multiplex (groom and huddle, Figure 1A) and

303   uniplex (grooming only, Figure 1B) affiliative networks (Table 3). Multiplex affiliation networks

304   had higher average edge-weight ($t(3) = 4.32$, $p = 0.023$; the average number of interactions per

305   social partner), clustering coefficient ($t(3) = 8.74$, $p = 0.003$), and modularity ($t(3) = 6.74$, $p =$

306   0.007; how much clustering is in the network) for all groups. Notably, although average edge-

307   weights in the multiplex networks were higher than uniplex networks, the predominant edge

308   weight in both networks was 1-2 grooming interactions (Figure S1). Multiplex affiliation

309   networks also consistently showed more kin bias (proportion kin, $t(3) = 5.74$, $p = 0.010$) and

310   reciprocity ($t(3) = 6.83$, $p = 0.006$) than uniplex networks. In contrast, both networks showed

311   associations between rank and affiliation (i.e., no difference in the tendency for grooming to be

312   directed up the hierarchy (Shapiro-Wilk: $W = 0.761$, $p = 0.048$; Wilcoxon signed rank $v = 0$, $p =$

313   0.125) or eigenvector centrality to be correlated with rank ($t(3) = -0.741$, $p = 0.51$)) but the

314   disparity in the ranks of the grooming partners was greater in the uniplex affiliation networks

315   compared to the multiplex networks ($t(3) = -10.534$, $p = 0.002$). Individual centrality metrics

316   generated from the multiplex networks were largely uncorrelated with metrics from the uniplex

317   networks (mean correlation strength $= 0.15$, $SD = 0.10$, Table S2).
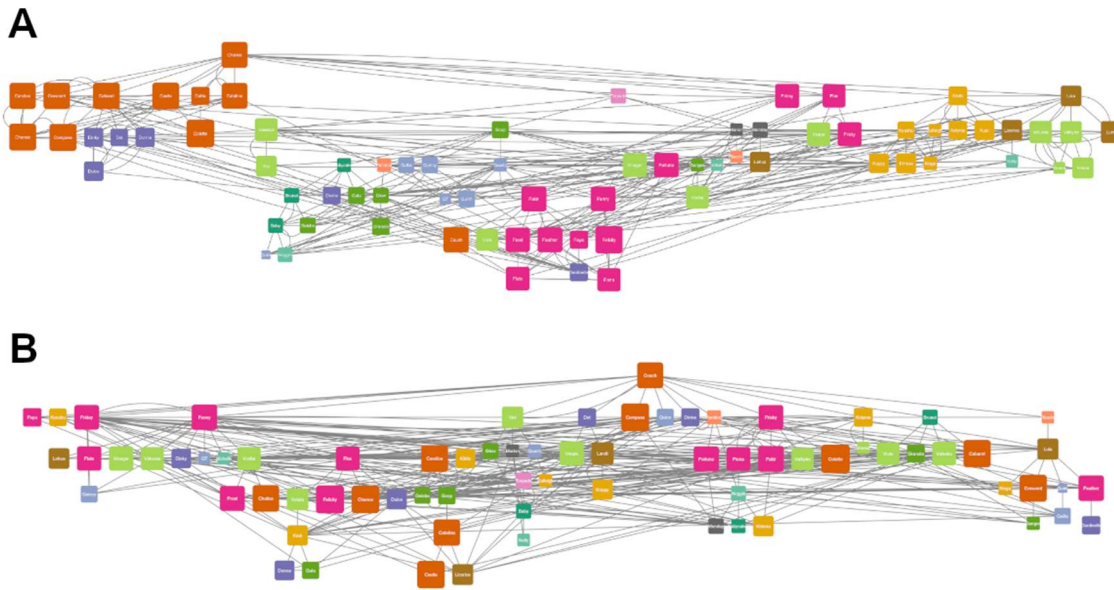
A

B

Figure 1. Example network graphs of (A) Multiplex affiliative network and (B) Uniplex affiliative network from social group A. Node color corresponds to matriline membership. Node size corresponds to rank (large nodes are high ranking). Curved lines reflect reciprocal edges. Layout is yFiles Tree Layout, Cytoscape.

Table 3: Network Level Metrics by Group

Group	A		B		C		D		
Network	Multi	Uni	Multi	Uni	Multi	Uni	Multi	Uni	Effect
Density	0.062	0.067	0.087	0.063	0.088	0.099	0.047	0.086	Multi = Uni
Modularity	0.688	0.272	0.515	0.281	0.473	0.219	0.698	0.290	Multi > Uni*
Eigenvector Centralization	0.807	0.654	0.659	0.589	0.710	0.636	0.829	0.538	Multi > Uni+
Avg Edge Weight	4.074	1.480	2.834	1.400	2.215	1.445	3.643	1.471	Multi > Uni*
Clustering Coefficient	0.269	0.157	0.249	0.147	0.270	0.201	0.261	0.185	Multi > Uni*
Reciprocity	0.534	0.239	0.466	0.200	0.400	0.233	0.573	0.215	Multi > Uni*
Proportion Kin	0.617	0.165	0.207	0.000	0.638	0.260	0.695	0.171	Multi > Uni*
Proportion Up Rank[a]	0.552	0.605	0.585	0.646	0.577	0.671	0.563	0.616	Multi = Uni
Rank Disparity	0.154	0.251	0.232	0.301	0.155	0.240	0.140	0.249	Multi < Uni*
Rank/Eigenvector centrality correlation	0.101	0.488	0.488	0.328	0.386	0.181	-0.230	0.308	Multi = Uni

Multi: Multidimensional affiliation network; Uni: Unidimensional affiliative network. Effect indicates the overall difference between multidimensional and unidimensional networks for all groups using a paired t-test. [a] Wilcoxon test. * $p < 0.05$, + $p < 0.1$

327   *Relationship Dimensionality and Biomarkers of Inflammation*

328   **IL-6.** The best fit models indicated that an individual's position in both the multiplex and uniplex

329   affiliative networks predicted levels of IL-6. Less connected individuals (i.e., low weighted

330   closeness) in the multiplex network had higher levels of IL-6 (Table 4). In contrast, more

331   connected individuals (i.e., high weighted closeness or weighted degree) in the uniplex network

332   exhibited higher levels of the pro-inflammatory cytokine IL-6. Multiplex weighted closeness was

333   not correlated with uniplex weighted degree ($r = -0.06$) but had a weak negative correlation with

334   uniplex closeness weight ($r = -0.23$). Uniplex weighted closeness and weighted degree were

335   highly correlated ($r = 0.84$, Table S2). Effects from the best fit model (Model 1) are shown in

336   Figure 2A-B.

337

338   **Table 4: Candidate Model Outputs**

	Model Parameters	**Model 1**	**Model 2**
IL-6	Multi Weighted Closeness	**-1.15** (-2.00 – -0.30)	**-1.35** (-2.19 – -0.51)
	Uni Weighted Closeness	**2.95** (1.21 – 4.69)	-
	Uni Weighted Degree	-	**0.041** (0.016 – 0.066)
	Intercept	1.14 (-1.28 – 3.56)	**2.72** (1.38 – 4.07)
	AIC	1520.65	1520.77
TNF-α	Multi Weighted Closeness	**-2.54** (-3.66 – -1.42)	**-2.07** (-3.22 – -0.93)
	Uni Betweenness	**0.005** (0.001 – 0.008)	-
	Uni Degree	-	**0.07** (0.02 – 0.12)
	Intercept	**6.74** (5.19 – 8.30)	**6.03** (4.24 – 7.82)
	AIC	2627.69	2628.74

339   β (95% CI). Bold values: $p < 0.05$. All models were run using a negative binomial distribution

340   and included a random effect of group. Multi: Multiplex affiliation network, Uni: Uniplex

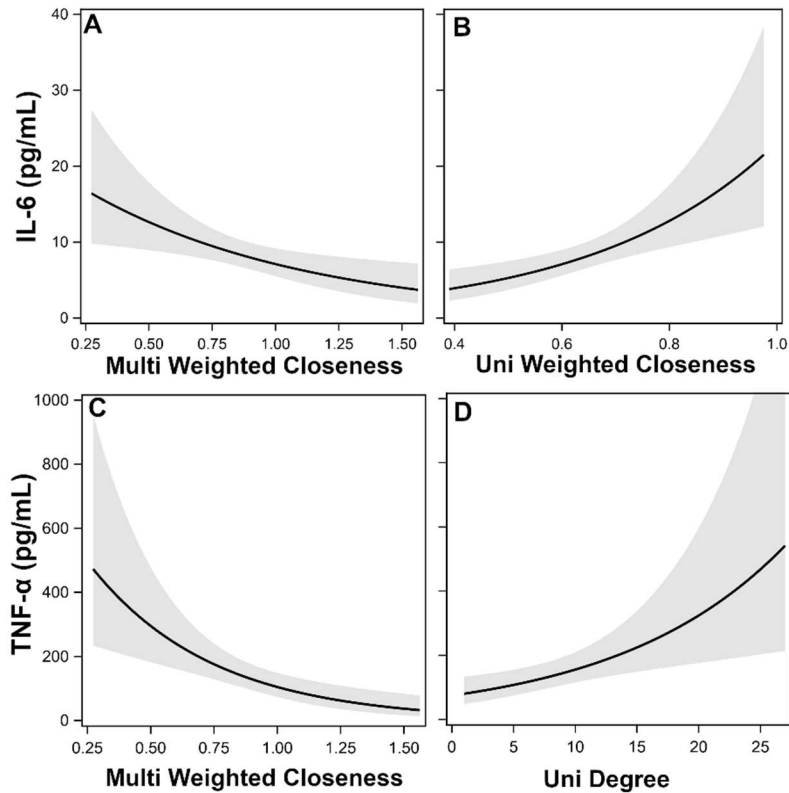341   affiliation network.

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Figure 2. Effects of affiliative centrality on cytokines. Effects of multiplex (Multi) weighted closeness (A) and uniplex (Uni) weighted closeness (B) on levels of IL-6 with 95% confidence intervals (Model 1). Effects of multiplex weighted closeness (C) and uniplex degree (D) on levels of TNFα with 95% confidence intervals (Model 2).

TNF-α. Levels of TNF-α were also predicted by network position in both the multiplex and uniplex affiliation networks. As with IL-6, lower connectedness (i.e., weighted closeness) in the multiplex grooming network and greater connectivity (i.e., betweenness or degree) in the uniplex affiliation network were associated with higher levels of TNF-α (Table 4). Multiplex weighted closeness was not correlated with uniplex degree (r = -0.08) but had a weak positive correlation with uniplex betweeness (r = 0.20). Uniplex betweenness and degree were highly correlated (r = 0.79, Table S2). Examination of the effects in the best fit model (Model 1) containing multiplex weighted closeness and uniplex betweenness revealed unexpectedly high predicted values of TNF-α (i.e., higher than 99% of the sample) and large confidence intervals. This is likely due to low variability in multiplex weighted closeness at high levels of uniplex betweenness (Figure

367 S3). Therefore, we show effects of the second-best model (Model 2) containing multiplex

368 weighted closeness and uniplex degree in Figure 2C-D.

Discussion

370   Social primates have a complex web of differentiated social relationships, which vary in

371 their structure and function. While strong affiliative social relationships are usually associated

372 with better health, less is known on how the multidimensionality or diversity of affiliative

373 behaviors exhibited in social relationships might impact health. We identified affiliative

374 relationships that were multiplex (animals affiliated using both grooming and huddling behavior)

375 versus uniplex (animals only groomed). Examination of these networks revealed that they

376 differed in topology, kinship, and associations with rank. Multiplex networks were more

377 modular, clustered, reciprocal, had higher average edge weights, were more strongly associated

378 with kinship, and less likely to be related to dominance rank than were uniplex affiliative

379 networks. The health impacts of these two networks differed as well, with females that were *less*

380 socially embedded in multiplex affiliative networks exhibiting higher levels of biomarkers of

381 inflammation (IL-6 and TNF-α), whereas females *more* socially connected in uniplex networks

382 exhibited higher levels of biomarkers of inflammation. These results suggest that grooming

383 which occurs in the context of multiplex affiliative relationships may result in health benefits

384 (i.e., reduced inflammation) while grooming occurring in uniplex affiliative relationships may

385 have potential costs.

386   Networks consisting of dyads with multiplex affiliative relationships showed differences

387 from uniplex affiliative networks in network topology, kinship, and associations with dominance.

388 Multiplex networks had structural characteristics consistent with strong bonds or supportive

389 affiliative relationships[13,58,59]. Specifically, interactions in the multiplex networks were more

390   likely to be reciprocal, frequent (i.e., higher edge-weight), clustered, and associated with kinship,

391   suggesting they are relationships that are regularly maintained and potentially more stable across

392   time[13,59]. Previous methods demonstrating that strong bonds enhance fitness, particularly those

393   using sociality indices, have also used multiple behaviors to assess relationship strength (e.g.,

394   grooming and proximity[7,17,18]). However, these methods rely on total duration or frequency of

395   affiliation to describe relationships rather than characterizing the breadth or dimensionality of the

396   relationships (e.g., dyads can have high DSI through grooming, proximity, or both). Similar to

397   strong bonds, multiplex affiliative relationships may improve health and fitness by buffering

398   individuals from the negative impacts of stress, improving predator detection, promoting

399   offspring survival, and improving social stability[10,11,60,61].

400   Also consistent with the literature on strong affiliative bonds, being well connected to

401   others was associated with biomarkers of better health. Specifically, the negative association

402   between weighted closeness and biomarkers of inflammation indicated that individuals that were

403   less embedded in the network (e.g., at the periphery of the group) may be at greater risk for

404   inflammation related diseases[24]. Closeness measures how close a node is to all other nodes in a

405   network (i.e., how many edges must be traversed to get to all other nodes) which means that

406   individuals with high closeness can readily reach many other individuals in the network. In other

407   words, they are highly embedded in this supportive network. The fact that the weighted version

408   of closeness was the best predictor suggests that it's not enough to just be highly embedded, but

409   frequent engagement is also required to reap the benefits of this connectedness. Notably,

410   huddling degree was not a predictor of inflammation adding support to the idea that there is

411   something unique about these multiplex relationships that is not represented by looking at

412   grooming or huddling relationships alone. Our results add to the literature suggesting that strong

413   bonds may improve fitness by altering endocrine and immune function[2,15,62,63]. Consistent with

414   this idea, Yang et al.[64] found in humans that socially integrated individuals (i.e., those with more

415   social connections across multiple domains) exhibited lower inflammation, whereas social strain

416   (e.g., higher levels of family criticism or demands) was associated with greater inflammation.

417   Given that familial and friend relationships tend to endure through extended periods, often

418   persisting over decades (in both humans and NHPs), these relationships may have an important

419   and long-lasting impact on health.

420   Uniplex affiliative relationships may reflect affiliative relationships that are more

421   transactional in nature[65]. The fact that uniplex relationships are less kin biased but likely to

422   occur between dyads of more disparate ranks suggests that these relationships may be more

423   related to grooming being used as a commodity in exchange for tolerance or support from higher

424   ranking animals. These relationships are likely more transactional in nature, reflecting a desire

425   to maintain peace/tolerance or used in a biological market exchange[48,49], rather than reflecting a

426   strong affiliative relationship. The positive association between females' connectedness in

427   uniplex affilation networks and biomarkers of inflammation suggests that uniplex affiliative

428   relationships may not be supportive on their own and instead are associated with increased

429   physiological costs, at least in the short term. Specifically, predictors of inflammation in the

430   uniplex affilation networks included degree, weighted degree, weighted closeness, and

431   betweenness. The network metrics in the uniplex network were more highly correlated with each

432   other and therefore it is difficult to identify which specific aspect of centrality in the network

433   might be driving these effects. However, collectively this group of candidate predictors indicates

434   that greater general connectedness (direct and indirect) was associated with increased

435   inflammation. Uniplex affiliative relationships are maintained through generally less frequent

436 interactions that are more likely to occur between animals of disparate ranks which may result in

437 greater uncertainty regarding the outcome of any given interaction. This uncertainty may be

438 stressful, and therefore have at least short-term physiological costs[66]. If these relationships are

439 more transactional in nature, reflecting a desire to maintain peace/tolerance or used in a

440 biological market exchange[48,49], then maintaining more of these transactional relationships may

441 result in increased stress, which if sustained can result in long-term physiological costs[2]. It is

442 possible that these short-term costs are actually investments that may manifest in future benefits

443 (e.g., tolerance, alliance support) that would offset these costs, yet this is difficult to test as the

444 "commodities" exchanged may be heterogeneous and the time-scale for market exchanges is

445 often unclear[67]. However, other work points to benefits of weak or economically based bonds to

446 survival and reproduction[6,17] (although see[7]). While these types of connections may have

447 ultimate fitness benefits (e.g., alliance support, increased access to food), this research suggests

448 they may also be associated with proximate costs.

449 Conclusion

450 Both humans and many species of NHPs engage in a complex interconnected system of

451 social interactions. Understanding the mechanisms by which social relationships impact health

452 and fitness remains a challenge. Decades of research has established that affiliative social

453 relationships can benefit health, however, the complexity and multidimensionality of

454 relationships has yet to be explored. By utilizing a network approach, we were able to

455 characterize two types of affiliative social relationships that differed in their network topology,

456 kin bias, associations with rank, and importantly their associations with biomarkers of

457 inflammation. Our research has indicated that features of multiplex affiliative relationships are

458 consistent with the concept of a strong supportive relationships and may support health and

459   fitness. In contrast, more transactional affiliative relationships (e.g., uniplex affiliation) may

460   incur short-term health costs yet may result in ultimate benefits through commodity exchange.

461   Still unclear is whether these effects are specific to the combination of behaviors used here (i.e.,

462   huddling and grooming), or if other affiliative behaviors (e.g., proximity) might provide similar

463   information. Further research into the dimensionality of relationships might reflect different

464   qualities or functions of relationships is needed. However, this complexity is important to

465   consider for understanding the mechanisms underlying the impact of social relationships on

466   human and NHP health.

467

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630

631   Figure Captions

632   Figure 1. Example network graphs of (A) Multiplex affiliative network and (B) Uniplex

633   affiliative network from social group A. Node color corresponds to matriline

634   membership. Node size corresponds to rank (large nodes are high ranking). Curved lines

635   reflect reciprocal edges. Layout is yFiles Tree Layout, Cytoscape.

636

637   Figure 2. Effects of affiliative centrality on cytokines. Effects of multiplex (Multi) weighted

638   closeness (A) and uniplex (Uni) weighted closeness (B) on levels of IL-6 with 95%

639   confidence intervals (Model 1). Effects of multiplex weighted closeness (C) and uniplex

640   degree (D) on levels of TNFα with 95% confidence intervals (Model 2).

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