ONLINE SUPPORTING INFORMATION

Preen oil composition of Pied Flycatchers is similar between partners but differs between sexes and breeding stages

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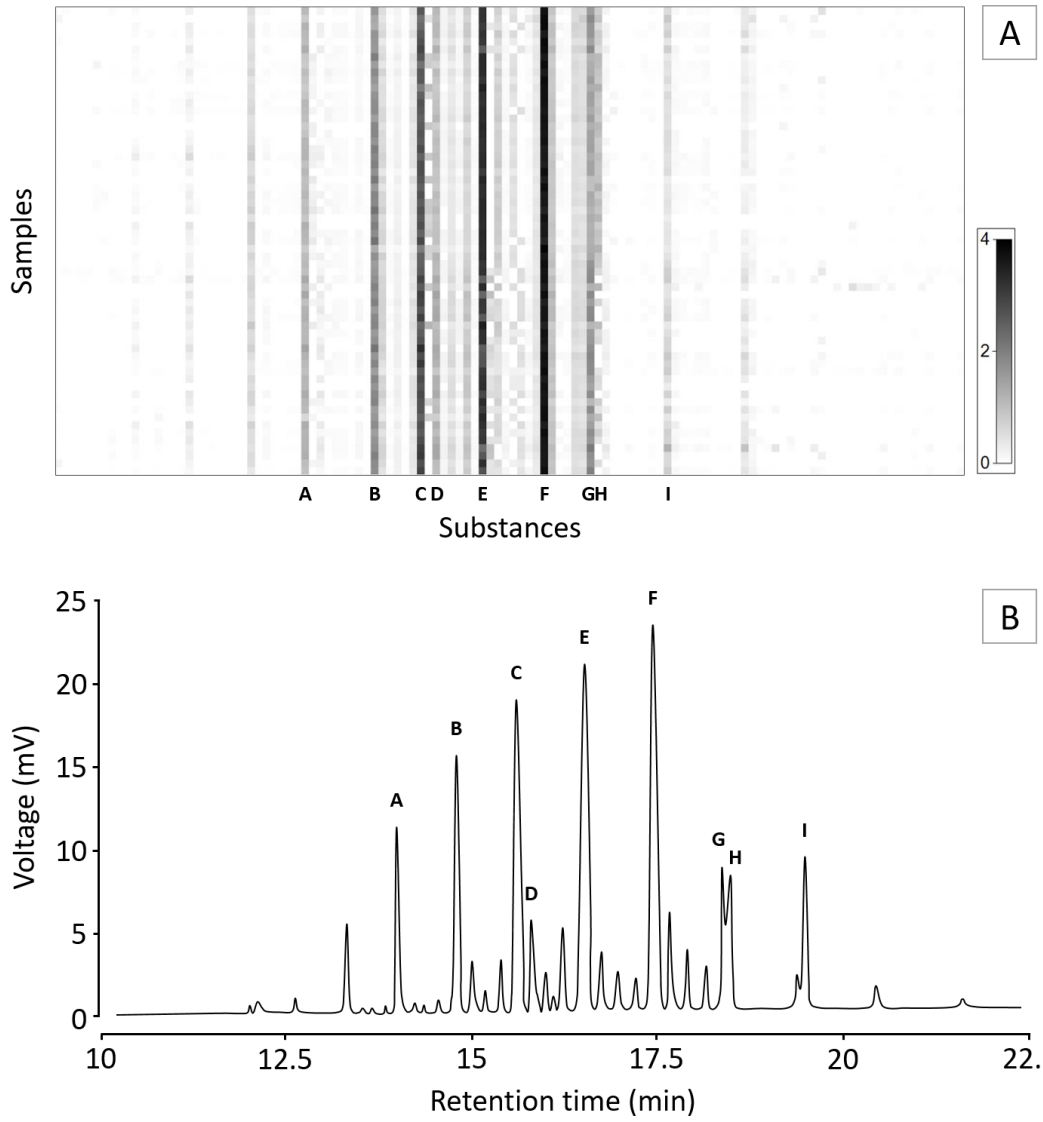

Figure S1. Chromatographic data from the GC-FID analysis of 77 preen oil samples of Pied Flycatchers. (a) Shadeplot showing the relative $\log_{10}$-transformed abundance of each substance (columns) in the samples (rows) used for the statistical analysis (only high-concentration samples are shown). (b) Representative chromatogram of the preen oil of a female Pied Flycatcher sampled during nestling rearing. Letters indicate the nine most abundant substances across all samples. Substance F was the most abundant substance across all samples and we used its abundance as a proxy of overall preen oil concentration in the samples.

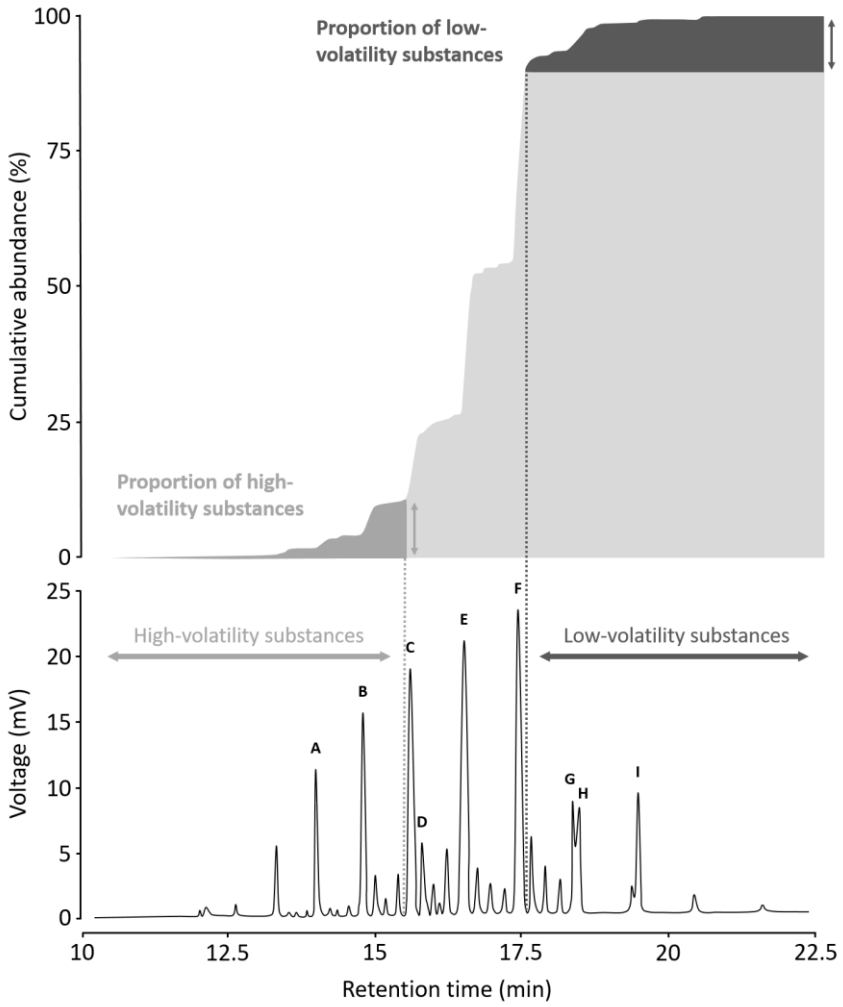

Figure S2. Calculation of the proportion of high-volatility and low-volatility substances. The proportion of high-volatility substances was measured as the proportion of abundance (i.e. chromatogram area) before peak C (i.e. substances with short retention time). The proportion of low-volatility substances was measured as the proportion of abundance after peak F (i.e. substances with long retention time). These thresholds (before peak C and after peak F) were selected to exclude the abundant central peaks while conserving sufficient portions of the chromatograms at each end. The analysis was repeated with another set of thresholds (earlier threshold for high-volatility substances, before peak B; later threshold for low-volatility substances, after peak H) and yielded similar results (see Table S9).

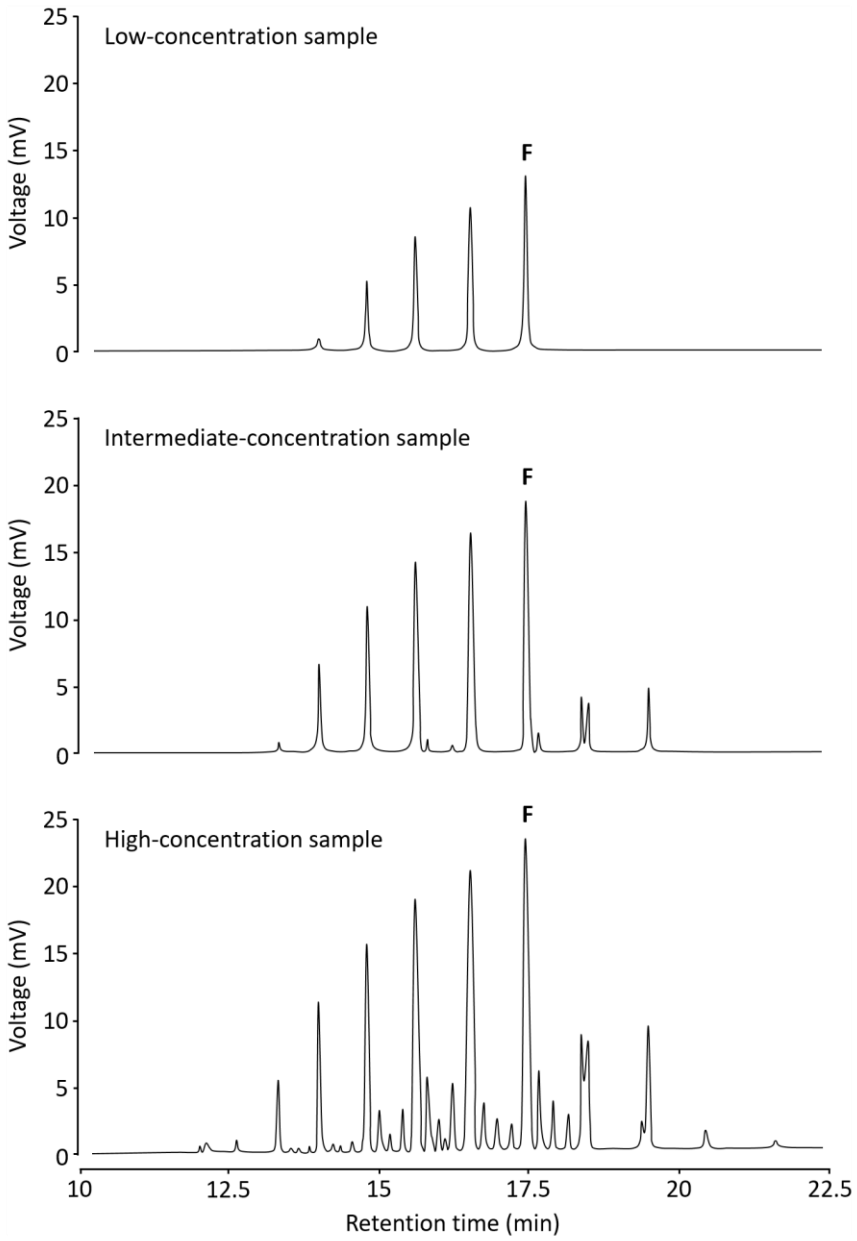

Figure S3. Concentration bias, illustrated by representative GC-FID chromatograms of three samples with varying overall concentrations of preen oil. Less substances are detected in samples with lower concentration, resulting in reduced chemical richness and diversity. Substance F was the most abundant substance across all samples and we used its abundance as a proxy of overall preen oil concentration in the samples.

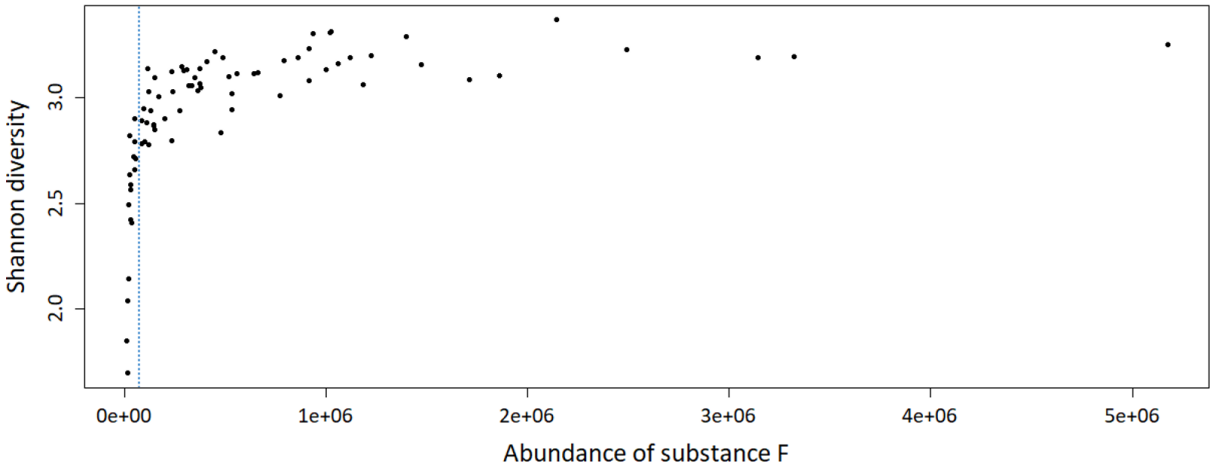

Figure S4. Chemical diversity (Shannon diversity index) in relation to the overall concentration in preen oil (abundance of substance F), revealing a concentration bias. Each dot represents a sample. Shannon diversity drops sharply below a certain threshold of concentration (abundance of substance F = 70000; indicated by the dashed line). Less substances are detected in low-concentration samples (abundance of substance F < 70000; left of the dashed blue line), so that their chromatogram reflects poorly their real chemical composition and possibly underestimates chemical richness and diversity). Low-concentration samples may introduce noise in our analysis and we therefore discarded samples below the threshold before running an additional analysis (our reduced dataset).

Table S1. Settings used for the integration of chromatographic data using the software GC Solutions (version 2.41) and for the alignment of chromatographic data using the *align_chromatograms* function of the *GCalignR* package in R.

	Parameter	**Value**
Peak integration	Width	1 sec
	Slope	500 uV/min
	Drift	0 uV/min
	Doubling time (T.DBL)	500 min
	Min. Area/Height	500 counts
Peak alignment	max_linear_shift	0 min
	max_diff_peak2mean	0.025 min
	min_diff_peak2peak	0.05 min

Table S2. Results from permutational multivariate analysis of variance (PERMANOVA) on the preen oil chemical composition of Pied Flycatchers including the low-concentration samples (complete dataset). (a) Effect of sex (fixed effect) within breeding pairs (random effect) sampled during nestling rearing (n = 34 samples from 17 pairs). (b) Effect of breeding stage (fixed effect) within individual females (random effect) sampled during both incubation and nestling rearing (n = 24 samples from 12 females).

	df	*SS*	*F* (pseudo)	*P* (perm)	Component of variation
(a) Sex and pair					
Sex	1	156.1	1.45	0.210	1.69
Pair	16	3606.5	2.09	**0.007**	7.67
Residuals	16	1721.7	—	—	10.37
(b) Breeding stage and individual					
Breeding stage	1	748.1	8.25	**0.004**	7.40
Individual	11	1214.1	1.22	0.270	3.16
Residuals	11	997.8	—	—	9.52

Analysis based on Bray-Curtis dissimilarities of log-transformed values. P-values were obtained using 9 999 permutations under a reduced model with type III (partial) sums of square (SS), and are indicated in bold if the effect is significant at α = 5%. Components of variation are 'pseudo' multivariate analogues of univariate variance components and were square-root-transformed to represent relative effect sizes in Bray-Curtis units (i.e. % of Bray-Curtis dissimilarity).

Table S3. Results from permutational multivariate analysis of variance (PERMANOVA) on the preen oil chemical composition of Pied Flycatchers considering only the nine most abundant substances. (a) Effect of sex (fixed effect) within breeding pairs (random effect) sampled during nestling rearing (complete dataset: n = 34 samples from 17 pairs; reduced dataset: n = 22 samples from 11 pairs). (b) Effect of breeding stage (fixed effect) within individual females (random effect) sampled during both incubation and nestling rearing (complete dataset: n = 24 samples from 12 females; reduced dataset: n = 16 samples from 8 females). The complete dataset includes all samples, whereas the reduced dataset includes only high-concentration samples.

	Complete dataset					**Reduced dataset**				
	df	*SS*	*F* (pseudo)	*P* (perm)	Component of variation	df	*SS*	*F* (pseudo)	*P* (perm)	Component of variation
(A) Sex and pair										
Sex	1	20.5	1.11	0.358	0.34	1	14.5	1.35	0.277	0.34
Pair	16	639.5	2.17	**0.008**	3.28	10	265.7	2.48	**0.034**	2.82
Residuals	16	295.2	—	—	4.30	10	107.1	—	—	3.27
(B) Breeding stage and individual										
Breeding stage	1	189.43	9.61	**0.005**	3.76	1	115.3	5.42	**0.033**	3.43
Individual	11	285.7	1.31	0.271	1.77	7	163.0	1.09	0.439	1.00
Residuals	11	216.7	—	—	4.44	7	149.0	—	—	4.61

Analysis based on Bray-Curtis dissimilarities of log-transformed values. *P*-values were obtained using 9 999 permutations under a reduced model with type III (partial) sums of square and are set in bold if the effect is significant at α = 5%. Components of variation are 'pseudo' multivariate analogues of univariate variance components and were square-root-transformed to represent relative effect sizes in Bray-Curtis units (i.e. % of Bray-Curtis dissimilarity).

Table S4. Results of generalized linear mixed models (GLMMs) investigating sex differences within breeding pairs in several chemical aspects of the preen oil of pied flycatchers: richness (number of substances), diversity (Shannon index), proportion of high-volatility substances and proportion of low-volatility substances. n = 24 samples from 12 pairs (12 females and 12 males).

Richness			
Fixed effect	β [95% CI]	P	Marginal R^2 [95% CI]
Sex (males)	–2.73 [–11.30, 5.80]	0.533	0.01 [0, 0.10]
Random effect	Variance (SD)	P	Repeatability [95% CI]
Pair	241.36 (15.54)	0.013	**0.71 [0.30, 0.92]**
Diversity			
Fixed effect	β [95% CI]	P	Marginal R^2 [95% CI]
Sex (males)	**–0.08 [–0.15, –0.01]**	0.051	0.07 [0, 0.29]
Random effect	Variance (SD)	P	Repeatability [95% CI]
Pair	0.01 (0.12)	0.011	**0.67 [0.21, 0.91]**
High-volatility			
Fixed effect	β [95% CI]	P	Marginal R^2 [95% CI]
Sex (males)	**–1.35 [–2.46, –0.20]**	0.029	0.21 [0.01, 0.53]
Random effect	Variance (SD)	P	Repeatability [95% CI]
Pair	0 (0)	1	0 [0, 0.60]
Low-volatility			
Fixed effect	β [95% CI]	P	Marginal R^2 [95% CI]
Sex (males)	0.03 [–0.83, 0.95]	0.950	0 [0, 0.13]
Random effect	Variance (SD)	P	Repeatability [95% CI]
Pair	1.61 (1.27)	0.037	**0.58 [0.06, 0.87]**

Fixed effects as well as repeatability was considered significant (α = 5%) if their 95% confidence interval did not include zero and are indicated in bold. β [95% CI]: Beta estimate and 95% confidence interval. SD: standard deviation. Repeatability: adjusted repeatability. P-values are indicated but were not used to assess significance. P-values of random effects are based on permutations.

Table S5. Results from permutational multivariate analysis of variance (PERMANOVA) examining the effects of factors on the preen oil chemical composition of pied flycatchers while controlling for the concentration in preen oil (abundance of the most abundant substance). (a) Effect of concentration and sex (fixed effects) within breeding pairs (random effect) sampled during nestling rearing (n = 34 samples from 17 pairs using the complete dataset; n = 22 samples from 11 pairs using the reduced dataset). (b) Effect of concentration and breeding stage (fixed effects) within individual females (random effect) sampled both during incubation and during nestling rearing (n = 24 samples from 12 females using the complete dataset; n = 16 samples from 8 females using the reduced dataset). The complete dataset includes all samples, whereas the reduced dataset includes only high-concentration samples.

	Complete dataset					**Reduced dataset**				
	df	*SS*	*F* (pseudo)	*P* (perm)	Component of variation	df	*SS*	*F* (pseudo)	*P* (perm)	Component of variation
(A) Sex and pair										
Concentration	1	560.2	3.06	**0.033**	3.33	1	189.5	2.05	0.093	2.10
Sex	1	193.3	1.80	0.138	2.25	1	191.0	3.80	**0.027**	3.65
Pair	16	3127.6	1.83	**0.019**	6.83	10	995.6	2.05	**0.034**	5.27
Residuals	15	1603.2	—	—	10.34	9	437.2	—	—	6.97
(B) Breeding stage and individual										
Concentration	1	252.2	2.65	0.055	2.56	1	164.5	1.97	0.145	2.25
Breeding stage	1	727.8	8.32	**0.006**	7.41	1	459.3	6.11	**0.021**	7.05
Individual	11	1107.6	1.15	0.342	2.66	7	635.5	1.21	0.342	2.94
Residuals	10	872.4	—	—	9.34	6	448.9	—	—	8.65

Analysis based on Bray-Curtis dissimilarities of log-transformed values. *P*-values were obtained using 9 999 permutations under a reduced model with type I (sequential) sums of square, and are set in bold if the effect is significant at α = 5%. Components of variation are 'pseudo' multivariate analogues of univariate variance components and were square-root-transformed to represent relative effect sizes in Bray-Curtis units (i.e. % of Bray-Curtis dissimilarity).

Table S6. Results from permutational multivariate analysis of variance (PERMANOVA) examining sex differences (fixed effect) within breeding pairs (random effect) on the preen oil chemical composition of pied flycatchers sampled during nestling rearing, while controlling for the temporal effects of sampling date (fixed effect) and time of day (fixed effect). n = 34 samples from 17 pairs using the complete dataset; n = 22 samples from 11 pairs using the reduced dataset. The complete dataset includes all samples, whereas the reduced dataset includes only high-concentration samples.

	Complete dataset					**Reduced dataset**				
	df	*SS*	*F* (pseudo)	*P* (perm)	Component of variation	df	*SS*	*F* (pseudo)	*P* (perm)	Component of variation
Sampling date	1	439.5	2.10	0.081	2.63	1	187.9	1.93	0.114	2.06
Sampling time	1	351.8	1.70	0.149	2.10	1	193.3	2.00	0.105	2.14
Sex	1	157.8	1.52	0.196	1.78	1	156.7	3.01	**0.045**	3.08
Pair	15	2975.6	1.91	**0.021**	7.11	9	806.4	1.72	0.071	4.59
Residuals	15	1559.5	—	—	10.20	9	469.0	—	—	7.22

Analysis based on Bray-Curtis dissimilarities of log-transformed values. *P*-values were obtained using 9 999 permutations under a reduced model with type I (sequential) sums of square, and are set in bold if the effect is significant at α = 5%. Components of variation are 'pseudo' multivariate analogues of univariate variance components and were square-root-transformed to represent relative effect sizes in Bray-Curtis units (i.e. % of Bray-Curtis dissimilarity).

Table S7. Results of generalized linear mixed models (GLMMs) investigating the effect of breeding stage within individuals in several chemical aspects of the preen oil of pied flycatchers: richness (number of substances), diversity (Shannon index), proportion of high-volatility substances and proportion of low-volatility substances. n = 16 samples from 8 individual females (8 during incubation, 8 during nestling rearing).

Richness			
Fixed effect	β [95% CI]	*P*	Marginal R^2 [95% CI]
Breeding stage (rearing)	1.12 [–18.00, 21.40]	0.913	0 [0, 0.30]
Random effect	Variance (SD)	*P*	Repeatability [95% CI]
Individual	0 (0)	1	0 [0, 0.68]
Diversity			
Fixed effect	β [95% CI]	*P*	Marginal R^2 [95% CI]
Breeding stage (rearing)	0.05 [–0.13, 0.24]	0.556	0.02 [0, 0.36]
Random effect	Variance (SD)	*P*	Repeatability [95% CI]
Individual	0 (0)	1	0 [0, 0.73]
High-volatility			
Fixed effect	β [95% CI]	*P*	Marginal R^2 [95% CI]
Breeding stage (rearing)	–1.12 [–2.72, 0.34]	0.200	0.06 [0, 0.35]
Random effect	Variance (SD)	*P*	Repeatability [95% CI]
Individual	2.42 (1.55)	0.134	0.49 [0, 0.89]
Low-volatility			
Fixed effect	β [95% CI]	*P*	Marginal R^2 [95% CI]
Breeding stage (rearing)	**1.84 [0.89, 2.94]**	0.004	0.43 [0.13, 0.74]
Random effect	Variance (SD)	*P*	Repeatability [95% CI]
Individual	0 (0)	1	0 [0, 0.69]

Fixed effects as well as repeatability was considered significant (α = 5%) if their 95% confidence interval did not include zero and are indicated in bold. β [95% CI]: Beta estimate and 95% confidence interval. SD: standard deviation. Repeatability: adjusted repeatability. P-values are indicated but were not used to assess significance. P-values of random effects are based on permutations.

Table S8. Results of the tests for homogeneity of multivariate dispersions (PERMDISP) to test for differences between sexes and breeding stages in dispersion (i.e. in deviations from centroid) in preen oil chemical composition. Non-significant differences indicate that the groups (females and males, incubation and nestling rearing period) have homogeneous dispersions.

	Complete dataset					**Reduced dataset**				
	Size	Mean	SE	*F*	*P* (perm)	Size	Mean	SE	*F*	*P* (perm)
Sex	—	—	—	0.69	0.52	—	—	—	0.35	0.58
Female	17	12.10	1.23	—	—	11	8.59	0.95	—	—
Male	17	10.55	1.40	—	—	11	7.86	0.79	—	—
Breeding stage	—	—	—	3.90	0.08	—	—	—	3.50	0.10
Incubation	12	10.28	1.33	—	—	8	9.68	1.53	—	—
Nestling rearing	12	7.21	0.80	—	—	8	6.61	0.59	—	—

Analysis based on Bray-Curtis dissimilarities of log-transformed values. *P*-values were obtained using 9 999 permutations and significance was assessed at α = 5%. SE: standard error.

Table S9. Results of generalized linear mixed models (GLMMs) on volatility using the alternative thresholds to measure the proportion of high-volatility and low-volatility substances (see Fig. S2). Results (β estimates and *P*-values) are consistent with the results obtained using the first threshold (Table S4 & S7).

(A) Sex and pair		
High-volatility (alternative threshold)		
Fixed effect	β	*P*
Sex (males)	**–1.06**	0.004
Random effect	Variance (SD)	
Pair	0 (0)	
Low-volatility (alternative threshold)		
Fixed effect	β	*P*
Sex (males)	0.20	0.618
Random effect	Variance (SD)	
Pair	1.15 (1.07)	
(B) Breeding stage and individual		
High-volatility (alternative threshold)		
Fixed effect	β	*P*
Breeding stage (rearing)	0.28	0.351
Random effect	Variance (SD)	
Individual	0.72 (0.84)	
Low-volatility (alternative threshold)		
Fixed effect	β	*P*
Breeding stage (rearing)	**1.24**	0.018
Random effect	Variance (SD)	
Individual	0 (0)	

Fixed effects were considered significant (α = 5%) if their 95% confidence interval does not include zero and are indicated in bold.